Proteomic Profiles of Adipose and Liver Tissues from an Animal Model of Metabolic Syndrome Fed Purple Vegetables

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Abstract: Metabolic Syndrome (MetS) is a complex disorder that predisposes an individual to Cardiovascular Diseases and type 2 Diabetes Mellitus. Proteomics and bioinformatics have proven to be an effective tool to study complex diseases and mechanisms of action of nutrients. We previously showed that substitution of the majority of carbohydrate in a high fat diet by purple potatoes (PP) or purple carrots (PC) improved insulin sensitivity and hypertension in an animal model of MetS (obese Zucker rats) compared to a control sucrose-rich diet. In the current study, we used TMT 10plex mass tag combined with LC-MS/MS technique to study proteomic modulation in the liver (n = 3 samples/diet) and adipose tissue (n = 3 samples/diet) of high fat diet-fed rats with or without substituting sucrose for purple vegetables, followed by functional enrichment analysis, in an attempt to elucidate potential molecular mechanisms responsible for the phenotypic changes seen with purple vegetable feeding. Protein folding, lipid metabolism and cholesterol efflux were identified as the main modulated biological themes in adipose tissue, whereas lipid metabolism, carbohydrate metabolism and oxidative stress were the main modulated themes in liver. We propose that enhanced protein folding, increased cholesterol efflux and higher free fatty acid (FFA) re-esterification are mechanisms by which PP and PC positively modulate MetS pathologies in adipose tissue, whereas, decreased de novo lipogenesis, oxidative stress and FFA uptake, are responsible for the beneficial effects in liver. In conclusion, we provide molecular evidence for the reported metabolic health benefits of purple carrots and potatoes and validate that these vegetables are good choices to replace other simple carbohydrate sources for better metabolic health.

Keywords: obesity; hypertension; insulin resistance; high fat diet; carrots; potatoes; proteomic analyses

1. Introduction

Metabolic Syndrome (MetS) is a complex disorder that predisposes an individual to type 2 diabetes (T2D) and Cardiovascular diseases (CVD). Insulin resistance (IR) is frequently identified as a leading factor in these pathologies [1]. Use of proteomic and bioinformatic tools in protein expression studies enables greater understanding of biological mechanisms of complex diseases and also mechanisms of action of drugs and/or nutrients [2,3]. Proteins are the final and active product of most of the genome and thus, their levels are the most accurate reflection of what is happening when gene expression is modulated. Poor correlation between mRNA and protein expression, attributed to impaired translation
efficiency [4], emphasizes the significance of directly determining protein abundance. Western blotting has been an effective tool for the study of protein expression for the last 30 years; however, it is limited by the size of the candidate pool that can be examined, giving an incomplete picture of the molecular phenotype.

In previous proteomic analyses, changes in the hepatic proteome in MetS, induced by high fat and high fructose diets in rodents [5,6], demonstrated modulation of proteins involved in glucose metabolism, lipid metabolism, oxidative stress and endoplasmic reticulum stress. The feeding of polyphenol-rich plants, including those high in a subclass described as anthocyanins, has been shown to modify the protein and/or mRNA expression of several genes known to be involved in the processes of lipid metabolism, inflammation and energy homeostasis in the liver and/or adipose tissues [7–13]. These changes were associated with an improvement in various metabolic risk factors including glucose tolerance, insulin sensitivity, hyperlipidemia, hyperinsulinemia and hepatic steatosis [7–13]. However, to our knowledge, there has yet to be a study that examined whole proteomic changes in response to anthocyanin-rich plant-supplemented diets. Such a study would provide an unbiased and comprehensive picture of the molecular mechanisms responsible for these plants’ biological activity.

We previously showed that the substitution of the majority of carbohydrate in a high fat diet, with purple carrots (PC) or purple potatoes (PP), for 8 weeks, improved insulin sensitivity and blood pressure compared to a control high fat sucrose-rich diet in a model of MetS, obese Zucker rats. PP were more effective in improving insulin sensitivity while PC were more effective on the blood pressure measures [14]. The current study aimed to examine the proteomic changes in the liver and adipose tissues of these animals using tandem mass tag (TMT 10plex) labelling combined with liquid chromatography tandem mass spectrometry (LC-MS/MS). This technique enables the concurrent identification and comparative quantitation of the peptides from 10 different samples. These profiles are then used to generate potential molecular mechanisms for the observed phenotypic changes induced by these vegetables (i.e., improvement in insulin sensitivity and blood pressure).

2. Materials and Methods

2.1. Experimental Design, Sample Collection and Tissue Homogenization

Liver and adipose tissue samples were collected from rats ad libitum fed 3 exact experimental modified high fat AIN-93M diets (Research Diets Inc., New Brunswick, NJ, USA) (n = 15 rats/diet) that only differed for the carbohydrate source for 8 weeks (Table 1). The control diet had sucrose whereas PP and PC diets had purple potatoes and purple carrots as main sources of carbohydrate as previously described in detail [14]. This protocol was approved by the Animal Care Committee of the University of Guelph (Animal Utilization Protocol #12R012) in accordance with the guidelines from the Canadian Council on Animal Care (CCAC). A subsample of frozen liver (n = 3 per diet group) and adipose tissues (n = 3 per diet group) were randomly selected and homogenized (Fast Prep® 24; MP biomedical, Santa Ana, CA, USA) using NP40 cell lysis buffer (Invitrogen, Camarillo, CA, USA) (3 volumes for adipose and 30 volumes for liver samples) supplemented with protease inhibitor cocktail and phenyl methyl sulfonyl fluoride (Sigma-Aldrich, St. Louis, MO, USA). The lysates were centrifuged at 5000 × g for 10 min at 4 °C [15,16]. Total protein content of the infranatant was determined using a BCA protein assay kit (Thermo Fisher, Rockford, IL, USA). The lysates were then sent to the SPARC BioCentre, SickKids Hospital (Toronto, ON, Canada) to perform the TMT labelling and LC-MS/MS analyses.
Table 1. Composition of the experimental diets.

| Component in g/kg Diet | Control | PP 1 | PC 2 |
|------------------------|---------|------|------|
| Casein (protein)       | 140     | 140  | 140  |
| L-Cystine              | 1.8     | 1.8  | 1.8  |
| Lard                   | 120     | 120  | 120  |
| Soybean Oil            | 40      | 40   | 40   |
| Maltodextrin 10        | 150     | 150  | 150  |
| Sucrose                | 450     | -    | 150  |
| Freeze dried baked purple potato | - | 450 | - |
| Freeze dried raw purple carrot | - | - | 300 |
| Cellulose, BW200       | 50      | 50   | 50   |
| Vitamin Mix v10037     | 10      | 10   | 10   |
| Mineral Mix s10022M    | 35      | 35   | 35   |
| Choline bitartrate     | 2.5     | 2.5  | 2.5  |

1 PP is high fat diet supplemented with purple potatoes; 2 PC is high fat diet supplemented with purple carrots.

2.2. Sample Preparation (Denaturation, Alkylation and Digestion) and TMT Labelling

The samples were solubilized with 1% Sodium dodecyl sulfate (SDS) and 8 M urea with sonication. The proteins were reduced in 1 mM dithiothreitol (DTT) for 1 h at 56 °C and the free cysteine residues were alkylated by incubating with iodoacetamide for 30 min protected from light at room temperature. The proteins were precipitated with 5 volumes of prechilled acetone overnight at −20 °C. The samples were centrifuged at 8000 × g for 10 min at 4 °C. The pellets were dried for 2–3 min before dissolving with triethylammonium bicarbonate (TEAB). The samples were then digested with trypsin 2.5 µg for 100 µg of protein overnight at 37 °C. Fifty micrograms of protein from each sample was labeled using 0.4 mg of TMT 10plex (ThermoFisher, Rockford, IL, USA) by incubating at room temperature for 1 h. The labeling reaction was stopped using 5% hydroxylamine. The peptides were mixed and the solvent removed under vacuum.

2.3. Liquid Chromatography and Tandem Mass Spectrometry (LC-MS/MS)

The peptides were analyzed on an Orbitrap analyzer (Q-Exactive, ThermoFisher, San Jose, CA, USA) outfitted with a nanospray source and EASY-nLC nano-LC system (ThermoFisher, San Jose, CA, USA). A 75 µm × 50 cm PepMax RSLC EASY-Spray column filled with 2 µM C18 beads (ThermoFisher, San Jose, CA, USA) was used to load the peptide mixture at a pressure of 800 Bar. Peptides were then subjected to a stepwise gradient elution over 240 min at a rate of 250 nL/min (0–4% Acetonitrile containing 0.1% Formic Acid over 2 min; 4–28% Acetonitrile containing 0.1% Formic Acid over 226 min, 28–95% Acetonitrile containing 0.1% Formic Acid over 2 min, constant 95% Acetonitrile containing 0.1% Formic Acid for 10 min). In the Q-Exactive mass spectrometer (ThermoFisher, San Jose, CA, USA), one MS full scan (525–1600 m/z) was performed with an automatic gain control (AGC) target of 1 × 10⁶ maximum ion injection time of 120 ms and a resolution of 35,000 with subsequent 15 data-dependent MS/MS scans with a resolution of 35,000, an AGC target of 1 × 10⁶, maximum ion time of 120 ms, and one microscan. The intensity threshold required to trigger a MS/MS scan was at an underfill ratio of 0.2%. In the higher energy collision dissociation (HCD) trap, normalized collision energy of 30 V was used for the fragmentation. The dynamic exclusion was applied with an exclusion period of 40 s [17].

2.4. Protein Identification and Quantitation

The MS/MS data was searched against the Rat UniProt database using Proteome Discoverer version 1.4 (ThermoFisher, San Jose, CA, USA) which also extracted the quantitation data from the 10 TMT tags. The data was imported into Scaffold Q+ (Proteome Software, Portland OR, USA) for label based quantitative analysis. Protein identifications were accepted if they contained at least 2 identified peptides above 95% tandem mass spectrometry confidence (with 0% decoy false discovery rate (FDR)).
Differentially expressed proteins were determined by applying $t$-Test with unadjusted significance level $p < 0.05$ corrected by Benjamini–Hochberg.

2.5. In-Silico Functional Analyses

We performed in silico functional analyses of the differentially expressed proteins to explore the biological meaning behind the modulation of expression of these proteins by the purple vegetable diets. The Database for Annotation, Visualization and Integrated Discovery (DAVID) [18] was used to perform functional enrichment analyses. The enriched (i.e., overrepresented) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and gene ontology (GO) terms biological processes component in the list of the differentially expressed proteins were identified. To account for multi-hypotheses testing, the $p$-values of the enrichment analyses were adjusted using Benjamini–Hochberg ($p < 0.05$).

3. Results and Discussion

3.1. Adipose Tissue Protein Expression

A total of 1944 proteins were identified in the adipose tissue of the rats fed the PP, the PC and the control diets (Supplemental Table S1), in which 85 and 224 proteins were differentially expressed with the PP and the PC diets respectively. 46 and 118 proteins were downregulated whereas 39 and 106 proteins were upregulated with the PP and the PC respectively (Tables 2 and 3). 3 KEGG pathways and 220 biological processes were enriched in the proteins list of the PP diet while 24 KEGG pathways and 405 biological processes GO terms were enriched in the proteins list of the PC diet (at Benjamini $p$ value < 0.05) (Supplemental Tables S2 and S5). Some of the enriched pathways and processes observed were mainly involved in lipid metabolism and cholesterol efflux with both diets and protein folding with the PP alone (Tables 4 and 5).
Table 2. Differentially expressed proteins with Purple Potatoes diet in adipose tissue.

| Differentially Expressed Proteins | Gene Name | Log2 Fold Change | Up- or Down-Regulated | p Value |
|-----------------------------------|-----------|------------------|------------------------|--------|
| Serum albumin precursor            | Alb       | −0.17            | down                   | 0.0001 |
| Serotransferrin precursor          | Tf        | −0.33            | down                   | 0.0001 |
| Fatty acid synthase                | Fasn      | −0.26            | down                   | 0.0001 |
| Myosin-9                           | Myh9      | −0.14            | down                   | 0.0001 |
| Alpha-1-macroglobulin precursor    | A1m       | 0.32             | up                     | 0.0001 |
| Fibrillin-1 isoform X1             | Fbn1      | −0.12            | down                   | 0.0001 |
| Filamin-A isoform X2               | Flna      | −0.06            | down                   | 0.0001 |
| Spectrin beta chain, non-erythrocytic 1 isoform X1 | SPTBN1 | 0.07 | up | 0.0001 |
| 78 kDa glucose-regulated protein precursor | Hspa5 | 0.3 | up | 0.0001 |
| Membrane primary amine oxidase     | Aox3      | 0.11             | up                     | 0.0001 |
| Calreticulin precursor             | Calr      | 0.33             | up                     | 0.0001 |
| Transketolase isoform X1           | Tkt       | −0.19            | down                   | 0.0001 |
| Endoplasmic reticulum              | Hsp90b1   | 0.33             | up                     | 0.0001 |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor | Itih4 | 0.12 | up | 0.0001 |
| Carboxylesterase 1D precursor      | Ces1d     | 0.38             | up                     | 0.0001 |
| Pyruvate kinase FKM isoform X2     | Pkm       | −0.11            | down                   | 0.0001 |
| Apolipoprotein A-I preproprotein   | Apoa1     | 0.26             | up                     | 0.0001 |
| Hemopexin precursor                | Hpox      | −0.03            | down                   | 0.0001 |
| Coflin-1                           | Cfl1      | −0.16            | down                   | 0.0001 |
| Vitamin D-binding protein precursor| Gc        | −0.14            | down                   | 0.0001 |
| Fibrinogen beta chain precursor    | Fgb       | 0.2              | up                     | 0.0001 |
| Myoferlin                          | Myoef     | −0.18            | down                   | 0.0001 |
| Hypoxia up-regulated protein 1 isoform X1 | Hyou1 | 0.38 | up | 0.0001 |
| Plastin-3 isoform X2               | Pls3      | 0.29             | up                     | 0.0001 |
| Complement factor B precursor      | Cfb       | −0.25            | down                   | 0.0001 |
| Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor | Cps1 | −0.38 | down | 0.0001 |
| Fibrinogen gamma chain isoform X1  | Fgg       | 0.22             | up                     | 0.0001 |
| Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2 isoform X1 | Rpm2 | 0.26 | up | 0.0001 |
| UDP-glucose:glycoprotein glucosyltransferase 1 precursor | Ugggt1 | 0.15 | up | 0.0001 |
| Protein disulfide-isomerase A6 precursor | Pdia6 | 0.31 | up | 0.0001 |
| Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1 precursor | Rpm1 | 0.22 | up | 0.0001 |
| Adipocyte plasma membrane-associated protein isoform X2 | Amap | 0.16 | up | 0.0001 |
| Acetyl-coa carboxylase 1           | Acaca     | −0.26            | down                   | 0.0001 |
| Apolipoprotein E precursor         | Apoe      | −0.4             | down                   | 0.0001 |
| Fibrinogen alpha chain isoform 2 precursor | Fga | 0.21 | up | 0.0001 |
| Catechol O-methyltransferase isoform X1 | Cmt | −0.21 | down | 0.0001 |
| Peroxiredoxin-5, mitochondrial precursor | Prdx5 | −0.29 | down | 0.0001 |
| Phosphoenolpyruvate carboxykinase, cytosolic [GTP] | Pck1 | 0.19 | up | 0.0001 |
| Differentially Expressed Proteins | Gene Name | Log2 Fold Change | Up- or Down-Regulated | p Value |
|----------------------------------|-----------|-----------------|-----------------------|---------|
| Coronin-1A isoform X1            | CORO1A    | −0.24           | down                  | 0.0001  |
| Hydroxymethylglutaryl-coa synthase, mitochondrial isoform X1 | Hmgs2 | −0.25           | down                  | 0.0001  |
| Complement component C7 isoform X1 | C7      | −0.25           | down                  | 0.0001  |
| Perilipin-2                      | Plin2     | −0.4            | down                  | 0.0001  |
| Galectin-3                       | Lgals3    | −0.44           | down                  | 0.0001  |
| Integrin alpha-M isoform X1      | Itgam     | −0.3            | down                  | 0.0001  |
| Brain acid soluble protein 1     | Basp1     | 0.19            | up                    | 0.0001  |
| Carbonyl reductase [NADPH] 1     | LOC102556347 | 0.27        | up                    | 0.0001  |
| Apolipoprotein C-II precursor     | Apoc2     | 0.46            | up                    | 0.0001  |
| Laminin subunit alpha-4 precursor | Lama4   | 0.1             | up                    | 0.0002  |
| Protein disulfide-isomerase A3 precursor | Pdia3 | 0.22            | up                    | 0.0002  |
| Cathepsin D precursor            | Ctsd      | −0.23           | down                  | 0.0002  |
| Macrophage mannose receptor 1 precursor | Mrc1  | −0.14           | down                  | 0.0003  |
| Filamin-B                        | Flnb      | 0.11            | up                    | 0.0003  |
| 3-ketoacyl-coa thiolase, mitochondrial | Acaa2 | −0.14           | down                  | 0.0003  |
| Chloride intracellular channel protein 1 | Clic1 | −0.15           | down                  | 0.0003  |
| Integrin beta-2 precursor        | Itgb2     | −0.26           | down                  | 0.0003  |
| Cystatin-B                       | Cstb      | −0.23           | down                  | 0.0003  |
| Von Willebrand factor A domain-containing protein 5A isoform X2 | LOC108348048 | −0.16     | down                  | 0.0003  |
| Apolipoprotein A-II isoform X1    | Apoa2     | 0.41            | up                    | 0.0003  |
| Neutral alpha-glucosidase AB isoform X1 | Ganab | 0.14            | up                    | 0.0004  |
| Transaldolase                    | Taldo1    | −0.14           | down                  | 0.0004  |
| Tissue-alpha-L-fucosidase precursor | Fucab  | 0.76            | up                    | 0.0004  |
| Phosphatidylethanolamine-binding protein 1 | Pep1 | 0.36            | up                    | 0.0004  |
| Apolipoprotein C-I precursor      | Apoc1     | 0.39            | up                    | 0.0005  |
| Protein disulfide-isomerase A4 precursor | Fdsd4 | 0.29            | up                    | 0.0006  |
| Selenium-binding protein 1 isoform X1 | LOC103689947 | −0.12      | down                  | 0.0006  |
| Heat shock 70 kDa protein 1A      | Hspa1b    | 0.12            | up                    | 0.0006  |
| Ester hydrolase c11orf54 homolog  | RGD1309534 | −0.15     | down                  | 0.0006  |
| Complement C3 precursor           | C3        | −0.15           | down                  | 0.0007  |
| Reticulocalbin-1 precursor        | Rcn1      | 0.29            | up                    | 0.0007  |
| Histidine-tRNA ligase, cytoplasmic | Harls | 0.27            | up                    | 0.0007  |
| Transmembrane glycoprotein NMB precursor | Gpnmmb | −0.3            | down                  | 0.0009  |
| Rho GDP-dissociation inhibitor 2 isoform X1 | Ardgdib | −0.22           | down                  | 0.0010  |
| Granulins isoform a precursor     | Gm        | −0.23           | down                  | 0.0011  |
| Betaine-homocysteine S-methyltransferase 1 | Blmt | −0.32           | down                  | 0.0012  |
| Plastin-2 isoform X2              | Lcp1      | −0.14           | down                  | 0.0012  |
| Transgelin-2 isoform X1           | Tagln2    | −0.15           | down                  | 0.0012  |
**Table 2.** Differentially Expressed Proteins with the Purple Potatoes Diet in adipose tissue.

| Differentially Expressed Proteins | Gene Name | Log2 Fold Change | Up- or Down-Regulated | p Value |
|-----------------------------------|-----------|-----------------|-----------------------|---------|
| Calnexin isoform X1               | Canx      | 0.13            | up                    | 0.0013  |
| Nucleolin                         | Ncl       | −0.13           | down                  | 0.0016  |
| Prothymosin alpha                 | Ptma      | −0.14           | down                  | 0.0016  |
| ATP synthase subunit d, mitochondrial | Atp5h  | −0.11           | down                  | 0.0017  |
| Alpha-1-acid glycoprotein precursor | Orm1   | −0.36           | down                  | 0.0017  |
| Perilipin-1 isoform X1            | Plin1     | 0.1             | up                    | 0.0018  |
| NAD(P)H-hydrate epimerase         | Naxe      | 0.18            | up                    | 0.0018  |
| Fructose-bisphosphate aldolase A isoform X2 | Aldoa | −0.09           | down                  | 0.0019  |
| Cysteine sulfenic acid decarboxylase isoform X1 | Csad | 0.12            | up                    | 0.0019  |

1 Log2 Fold Change by Category (Purple Potatoes/Control); 2 p value of the t-test less than 5% Benjamini–Hochberg threshold (0.0022).

**Table 3.** Differentially expressed proteins with the Purple Carrots Diet in adipose tissue.

| Differentially Expressed Proteins | Gene Name | Log2 Fold Change | Down- or Up-Regulated | p Value |
|-----------------------------------|-----------|-----------------|-----------------------|---------|
| Serum albumin precursor           | Alb       | −0.19           | down                  | 0.0001  |
| Serotransferrin precursor         | Tf        | −0.22           | down                  | 0.0001  |
| Fatty acid synthase               | Fasn      | −0.15           | down                  | 0.0001  |
| Myosin-9                          | Myh9l1    | −0.07           | down                  | 0.0001  |
| Elongation factor 1-alpha 1       | Efia1     | −0.11           | down                  | 0.0001  |
| Filamin-A isoform X2              | Flna      | −0.09           | down                  | 0.0001  |
| Alpha-enolase                     | Eno1      | −0.15           | down                  | 0.0001  |
| Ribosome-binding protein 1 isoform X4 | Rlp1 | −0.16           | down                  | 0.0001  |
| Plastin-2 isoform X2              | Lcp1      | −0.17           | down                  | 0.0001  |
| Aldehyde dehydrogenase, mitochondrial precursor | Aldh2 | −0.16           | down                  | 0.0001  |
| Collagen alpha-1 (XIV) chain precursor | Col14a1 | −0.44           | down                  | 0.0001  |
| ATP-citrate synthase isoform X1   | Acly      | −0.26           | down                  | 0.0001  |
| Glutamate dehydrogenase 1, mitochondrial precursor | Mrc1 | −0.14           | down                  | 0.0001  |
| Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor | Cps1 | −0.75           | down                  | 0.0001  |
| Heterogeneous nuclear ribonucleoprotein U | Hnrnpu | −0.2            | down                  | 0.0001  |
| Serine protease inhibitor A3N     | Serpina3n | −0.27           | down                  | 0.0001  |
| Decorin isoform X1                | Den       | −0.4            | down                  | 0.0001  |
| Glutathione S-transferase alpha-3 | Gsta1     | −0.27           | down                  | 0.0001  |
| Prolargin isoform X3              | Prelp     | −0.29           | down                  | 0.0001  |
| 3-ketoacyl-coa thiolase, mitochondrial | Acac2 | −0.29           | down                  | 0.0001  |
## Table 3. Cont.

| Defferentially Expressed Proteins | Gene Name | Log2 Fold Change $^1$ | Down- or Up-Regulated | $p$ Value $^2$ |
|-----------------------------------|-----------|------------------------|-----------------------|---------------|
| Acetyl-coa carboxylase 1          | Acaca     | −0.18                  | down                  | 0.0001        |
| Aspartate aminotransferase, mitochondrial | Got2    | −0.21                  | down                  | 0.0001        |
| Heterogeneous nuclear ribonucleoprotein K isoform X2 | Hnrnpk  | −0.18                  | down                  | 0.0001        |
| ATP synthase subunit d, mitochondrial | Atp5h   | −0.14                  | down                  | 0.0001        |
| Catechol O-methyltransferase isoform X1 | Comt     | −0.34                  | down                  | 0.0001        |
| Nucleolin                         | Ncl       | −0.32                  | down                  | 0.0001        |
| Hydroxymethylglutaryl-coa synthase, mitochondrial isoform X1 | Hmgcs2   | −0.47                  | down                  | 0.0001        |
| Complement component C7 isoform X1 | C7       | −0.21                  | down                  | 0.0001        |
| Galectin-3                        | Lgals3    | −0.34                  | down                  | 0.0001        |
| Biglycan precursor                | Bgn       | −0.24                  | down                  | 0.0001        |
| Granulins isoform a precursor     | Grn       | −0.33                  | down                  | 0.0001        |
| Ezrin                             | Ezr       | −0.24                  | down                  | 0.0001        |
| Nucleophosmin                     | Npm1      | −0.33                  | down                  | 0.0001        |
| Elongation factor Tu, mitochondrial precursor | Tufn     | −0.12                  | down                  | 0.0001        |
| Beta-2-glycoprotein 1 precursor   | Apol1     | −0.37                  | down                  | 0.0001        |
| Betaine-homocysteine S-methyltransferase 1 | Bmnt     | −0.69                  | down                  | 0.0001        |
| Obg-like atpase 1                 | Ola1      | −0.14                  | down                  | 0.0001        |
| Glutathione S-transferase Mu 1    | Gstm1     | −0.62                  | down                  | 0.0001        |
| High mobility group box 1 like    | Hmg1l1    | −0.4                   | down                  | 0.0001        |
| Alcohol dehydrogenase 1           | Adh1      | −0.75                  | down                  | 0.0001        |
| Fatty acid-binding protein, liver | Fabp1     | −0.77                  | down                  | 0.0001        |
| Von Willebrand factor A domain-containing protein 5A isoform X2 | LOC108348048 | −0.17               | down                  | 0.0001        |
| Serine/threonine-protein kinase N3 | Pkn3      | −0.26                  | down                  | 0.0001        |
| Heterogeneous nuclear ribonucleoprotein M isoform b | Hnrnpm  | −0.22                  | down                  | 0.0001        |
| Argininosuccinate synthase isoform X1 | Ass1    | −0.53                  | down                  | 0.0001        |
| Fructose-bisphosphate aldolase B  | Aldob     | −0.65                  | down                  | 0.0001        |
| LIM and senescent cell antigen-like-containing domain protein 1 | Limd1    | −0.17                  | down                  | 0.0001        |
| Arginase-1                        | Arg1      | −0.5                   | down                  | 0.0001        |
| Sorbitol dehydrogenase            | Sorid     | −0.31                  | down                  | 0.0001        |
| Carbonic anhydrase 3 isoform X1    | Car3      | 0.15                   | up                    | 0.0001        |
| Vimentin                          | Vim       | 0.23                   | up                    | 0.0001        |
| Long-chain-fatty-acid-coa ligase 1 isoform X1 | Acs1l   | 0.12                   | up                    | 0.0001        |
| Alpha-1-macroglobulin precursor   | Pzp       | 0.21                   | up                    | 0.0001        |
| Fibrillin-1 isoform X1             | Fbn1      | 0.15                   | up                    | 0.0001        |
| Complement C3 precursor           | C3        | 0.14                   | up                    | 0.0001        |
| Spectrin beta chain, non-erythrocytic 1 isoform X1 | Sptbn1  | 0.1                    | up                    | 0.0001        |
| Gene Name | Log2 Fold Change | Down- or Up-Regulated | p Value |
|-----------|------------------|-----------------------|---------|
| Plec      | 0.06             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
| Aoc3      | 0.21             | up                    | 0.0001  |
| Relsat    | 0.17             | up                    | 0.0001  |
| Col6a3    | 0.12             | up                    | 0.0001  |
| Vcl       | 0.12             | up                    | 0.0001  |
Table 3. Cont.

| Defferntially Expressed Proteins | Gene Name             | Log2 Fold Change | Down- or Up-Regulated | p Value  |
|---------------------------------|-----------------------|-----------------|-----------------------|----------|
| 1-acyl-sn-glycerol-3-phosphate acyltransferase gamma | Agpat3                | 0.18            | up                    | 0.0001   |
| GNAS isoform GNASL              | Gnas                  | 0.22            | up                    | 0.0001   |
| Chloride intracellular channel protein 1 | Clic1                | −0.15           | down                  | 0.0001   |
| Neprilysin isoform X1           | Mme                   | 0.24            | up                    | 0.0001   |
| Creatine kinase B-type          | Ckb                   | −0.15           | down                  | 0.0001   |
| Protein S100-B isoform X1       | S100b                 | 0.17            | up                    | 0.0001   |
| Fibrinogen beta chain precursor | Fgb                   | 0.14            | up                    | 0.0001   |
| Calumenin isoform a precursor   | Calu                  | 0.15            | up                    | 0.0001   |
| T-complex protein 1 subunit zeta| Cct6a                 | −0.12           | down                  | 0.0001   |
| Hepatoma-derived growth factor  | Hdgf                  | −0.3            | down                  | 0.0001   |
| Transaldolase                   | Taldo1                | −0.13           | down                  | 0.0002   |
| Sorbin and SH3 domain-containing protein 2 | Sorbs2             | 0.29            | up                    | 0.0002   |
| Fibrinogen gamma chain isoform X1 | Fgg                   | 0.14            | up                    | 0.0002   |
| Dysferlin                       | Dysf                  | 0.16            | up                    | 0.0002   |
| Aminoacyl tRNA synthase complex-interacting multifunctional protein 1 | Aimp1                | −0.32           | down                  | 0.0002   |
| Apolipoprotein C-III precursor  | Apc3                  | 0.25            | up                    | 0.0002   |
| Heat shock 70 kDa protein 1A    | Hspa1b                | 0.11            | up                    | 0.0002   |
| Transmembrane protein 43        | Tmem43                | 0.14            | up                    | 0.0002   |
| Monoglyceride lipase isoform X1 | Mgll                  | 0.14            | up                    | 0.0002   |
| Apolipoprotein A-IV precursor   | Apos4                 | 0.15            | up                    | 0.0002   |
| Alcohol dehydrogenase [NADP(+) | Akr1a1                | −0.13           | down                  | 0.0002   |
| Glucose-6-phosphate isomerase   | Gpi                   | 0.13            | up                    | 0.0002   |
| Lumican precursor               | Lum                   | −0.18           | down                  | 0.0003   |
| Glutamine synthetase            | Guls                  | −0.22           | down                  | 0.0003   |
| PDZ and LIM domain protein 1    | Pdlim1                | 0.25            | up                    | 0.0003   |
| Filamin-B                       | Flnb                  | 0.1             | up                    | 0.0003   |
| Legumain precursor              | Lgmn                  | −0.17           | down                  | 0.0003   |
| RNA-binding protein FUS isoform X1 | Fus                 | −0.2            | down                  | 0.0003   |
| Septin-9 isoform 2              | Sept9                 | −0.17           | down                  | 0.0003   |
| Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial | Aldh4a1               | −0.32           | down                  | 0.0003   |
| Cadherin-13 precursor           | Cdht13                | 0.25            | up                    | 0.0003   |
| Apolipoprotein C-II precursor   | Apc2                  | 0.34            | up                    | 0.0003   |
| Protein-glutamine gamma-glutamyltransferase 2 | Tgm2             | −0.3            | down                  | 0.0003   |
| Glutathione S-transferase Mu 2  | Gstmu2                | −0.25           | down                  | 0.0004   |
| 60S ribosomal protein L5        | Rpl5                  | −0.18           | down                  | 0.0004   |
Table 3. Cont.

| Defferntially Expressed Proteins | Gene Name            | Log2 Fold Change 1 | Down- or Up-Regulated | p Value 2 |
|-----------------------------------|----------------------|--------------------|------------------------|-----------|
| Transketolase isoform X1          | Tkt                  | −0.1               | down                   | 0.0005    |
| Synapse-associated protein 1 isoform X1 | Syap1  | 0.2              | up                     | 0.0005    |
| Sulfated glycoprotein 1 isoform X1 | Psap                | −0.32              | down                   | 0.0005    |
| Camp-dependent protein kinase type II-beta regulatory subunit | Prkar2b | 0.15              | up                     | 0.0005    |
| Proliferation-associated protein 2G4 | Pa2g4              | −0.27              | down                   | 0.0005    |
| L-lactate dehydrogenase A chain isoform X1 | Ldha     | −0.14              | down                   | 0.0005    |
| Unconventional myosin-1c           | Myo1c               | 0.07               | up                     | 0.0006    |
| Prelamin-A/C                       | Lmna                | 0.1                | up                     | 0.0006    |
| Phosphoserine aminotransferase     | Psat1               | 0.15               | up                     | 0.0006    |
| Isocitrate dehydrogenase [NADP, mitochondrial precursor] | Idh2       | −0.23              | down                   | 0.0006    |
| Reticulin-4                        | Rtn4                | 0.18               | up                     | 0.0006    |
| Transmembrane glycoprotein NMB precursor | Gpnmb         | −0.27              | down                   | 0.0006    |
| Nucleobindin-1 isoform X1          | Nuch1               | 0.13               | up                     | 0.0006    |
| Retinol dehydrogenase 11 precursor | Rdh11              | 0.28               | up                     | 0.0006    |
| Poly [ADP-ribose] polymerase 3     | Parp3               | −0.19              | down                   | 0.0007    |
| Hsc70-interacting protein          | St13                | 0.11               | up                     | 0.0007    |
| 40S ribosomal protein S19          | Rps19               | −0.23              | down                   | 0.0007    |
| Alpha-actinin-4                    | Actn4               | 0.08               | up                     | 0.0007    |
| Serine hydroxymethyltransferase, cytosolic | SlmA               | −0.25              | down                   | 0.0008    |
| Coflin-1                           | Cft1                | −0.12              | down                   | 0.0009    |
| Lamin-B1                           | Lmb1                | 0.17               | up                     | 0.0010    |
| Heterogeneous nuclear ribonucleoprotein A3 isoform a | Hurnpa3 | −0.26              | down                   | 0.0010    |
| Polymerase I and transcript release factor | Ptf1          | 0.12               | up                     | 0.0010    |
| Ras gtpase-activating-like protein IQGAP1 | Iqgap1      | −0.07              | down                   | 0.0011    |
| Probable ATP-dependent RNA helicase DDX5 isoform X1 | Ddx5       | −0.14              | down                   | 0.0011    |
| Eukaryotic initiation factor 4A-II isoform X1 | Eif4a2   | 0.12               | up                     | 0.0011    |
| Moesin                             | Msn                 | −0.14              | down                   | 0.0012    |
| Ribonuclease UK14                   | Rida                | −0.32              | down                   | 0.0012    |
| Dynactin subunit 2                 | Dctn2               | 0.1                | up                     | 0.0012    |
| Splicing factor U2AF 65 kDa subunit isoform X1 | U2af2 | −0.18              | down                   | 0.0013    |
| Annexin A1 isoform X2              | Anxa1               | 0.11               | up                     | 0.0013    |
| ATP synthase subunit O, mitochondrial precursor | Atp5o       | −0.13              | down                   | 0.0014    |
| Uncharacterized protein LOC315963   | RGD1310507          | −0.14              | down                   | 0.0014    |
| Coagulation factor XIII A chain    | Fl3a1               | 0.16               | up                     | 0.0014    |
| 1-acylglycerol-3-phosphate O-acyltransferase ABHD5 | Abhd5       | 0.16               | up                     | 0.0014    |
| Defferentially Expressed Proteins                                      | Gene Name    | Log2 Fold Change | Down- or Up-Regulated | p Value  |
|----------------------------------------------------------------------|--------------|------------------|-----------------------|----------|
| Receptor of activated protein C kinase 1                            | Rack1        | −0.16            | down                  | 0.0015   |
| Ethylmalonyl-coa decarboxylase isoform X2                          | Echdc1       | 0.15             | up                    | 0.0015   |
| Peptidyl-yl cis-trans isomerase FKB9 precursor                      | Fkbp9        | 0.2              | up                    | 0.0015   |
| Glutathione S-transferase Mu 5                                      | Gcl2         | −0.43            | down                  | 0.0016   |
| ATP synthase-coupling factor 6, mitochondrial isoform X2           | Atp5j        | −0.13            | down                  | 0.0016   |
| Epididymal secretory protein E1 precursor                           | Npc2         | −0.15            | down                  | 0.0016   |
| Glycerol-3-phosphate acyltransferase 3 isoform X1                   | Gap3         | 0.27             | up                    | 0.0016   |
| 60S ribosomal protein L4                                            | Rpl4         | −0.13            | down                  | 0.0017   |
| Carbonyl reductase [NADPH] 1                                        | LOC102556347 | 0.24             | up                    | 0.0017   |
| Transmembrane protein 120A                                          | Tmem120a     | 0.33             | up                    | 0.0017   |
| Annexin A5                                                          | Anxa5        | 0.12             | up                    | 0.0019   |
| Trifunctional enzyme subunit alpha, mitochondrial precursor         | Hadha        | −0.08            | down                  | 0.0021   |
| Sorbin and SH3 domain-containing protein 1 isoform X6              | Sorbs1       | 0.16             | up                    | 0.0021   |
| Long-chain fatty acid transport protein 3 precursor                 | Scl27a3      | 0.22             | up                    | 0.0021   |
| Ceruloplasmin isoform 1 precursor                                   | Cp           | −0.08            | down                  | 0.0022   |
| Heterogeneous nuclear ribonucleoproteins C1/C2-like isoform X5     | LOC100911576 | −0.13            | down                  | 0.0022   |
| Peroxisomal bifunctional enzyme                                     | Ethadh       | −0.29            | down                  | 0.0022   |
| Fructose-1,6-bisphosphatase 1                                       | Fbp1         | −0.52            | down                  | 0.0024   |
| Aconitate hydratase, mitochondrial precursor                        | Aco2         | 0.08             | up                    | 0.0025   |
| General vesicular transport factor p115 isoform X1                  | Uso1         | 0.14             | up                    | 0.0025   |
| Antigen-presenting glycoprotein CD1d precursor                      | Cd1d1        | 0.18             | up                    | 0.0025   |
| Bifunctional glutamate/proline-tRNA ligase isoform X1               | Eprs         | −0.12            | down                  | 0.0027   |
| Alpha-2-HS-glycoprotein precursor                                   | Aleg         | −0.27            | down                  | 0.0027   |
| Macrophage mannose receptor 1 precursor                             | Mrx1         | −0.17            | down                  | 0.0028   |
| Peptidyl-yl cis-trans isomerase B precursor                         | Ppib         | −0.17            | down                  | 0.0028   |
| 40S ribosomal protein S9                                            | LOC103689992 | −0.13            | down                  | 0.0028   |
| Aldehyde dehydrogenase family 8 member A1                           | Aldh8a1      | −0.8             | down                  | 0.0028   |
| Erlin-2 isoform X1                                                   | Erlin2       | 0.1              | up                    | 0.0028   |
| Peroxiredoxin-5, mitochondrial precursor                            | Prdx5        | −0.18            | down                  | 0.0029   |
| Pantetheinase precursor                                             | Vmn1         | 0.24             | up                    | 0.0029   |
| Adenosylhomocysteinase                                              | Ahcy         | −0.18            | down                  | 0.0030   |
| 3-oxo-5-beta-steroid 4-dehydrogenase                               | Akr1d1       | −0.44            | down                  | 0.0030   |
| Septin-11                                                           | Sept11       | −0.14            | down                  | 0.0032   |
| Cathepsin D precursor                                               | Clsd         | −0.16            | down                  | 0.0033   |
| ATP synthase subunit delta, mitochondrial isoform X1                | Atp5d        | 0.1              | up                    | 0.0033   |
Table 3. Cont.

| Defferentially Expressed Proteins | Gene Name | Log2 Fold Change | Down- or Up-Regulated | p Value |
|-----------------------------------|-----------|------------------|-----------------------|---------|
| Coronin-1A isoform X1              | Coro1A    | −0.14            | down                  | 0.0034  |
| Calcium-binding mitochondrial carrier protein Aralar2 isoform X1 | Slc25a13 | −0.33            | down                  | 0.0034  |
| Annexin A6                        | Anxa6     | 0.08             | up                    | 0.0034  |
| 40S ribosomal protein S15          | Rps15     | 0.23             | up                    | 0.0034  |
| Mitochondrial dicarboxylate carrier | Slc25a10 | −0.12            | down                  | 0.0035  |
| Serum deprivation-response protein | Sdpr      | 0.12             | up                    | 0.0035  |
| Ras-related protein Rab-2A        | Rab2a     | 0.12             | up                    | 0.0035  |
| Platelet endothelial cell adhesion molecule precursor | Pecam1 | 0.17             | up                    | 0.0036  |
| Glyceraldehyde-3-phosphate dehydrogenase | Gapdh | −0.09            | down                  | 0.0038  |
| Peptidyl-prolyl cis-trans isomerase A | LOC100360977 | −0.14          | down                  | 0.0039  |
| Actin-related protein 2/3 complex subunit 1B | Arpc1b | −0.17            | down                  | 0.0040  |
| Thiosulfate sulfurtransferase      | Tst       | −0.2             | down                  | 0.0040  |
| Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 | Gnb1 | 0.14             | up                    | 0.0040  |
| Phenylalanine-4-hydroxylase        | Pah       | −0.4             | down                  | 0.0046  |
| Talin-1                           | Tn1       | −0.05            | down                  | 0.0048  |
| 60S ribosomal protein L30          | Rpl30     | −0.14            | down                  | 0.0048  |
| Erythrocyte band 7 integral membrane protein | Stom | 0.26             | up                    | 0.0048  |
| Camp-dependent protein kinase catalytic subunit alpha | Prkaca | 0.23             | up                    | 0.0050  |
| Calcineurin B homologous protein 1 | Cip1    | 0.15             | up                    | 0.0050  |
| Trifunctional enzyme subunit beta, mitochondrial isoform X2 | Hadhb | −0.1             | down                  | 0.0052  |
| Transmembrane 9 superfamily member 3 isoform X2 | Tmn9f3 | −0.22            | down                  | 0.0052  |
| Peroxiredoxin-1                   | Prdx1     | −0.12            | down                  | 0.0053  |
| UDP-glucuronosyltransferase 2B2 precursor | Ugt2b | −0.64            | down                  | 0.0053  |
| Carbonyl reductase [NADPH] 3       | Cbr3      | 0.14             | up                    | 0.0055  |
| Guanlyate-binding protein 4 isoform X1 | LOC685067 | 0.15             | up                    | 0.0056  |
| Creatine kinase M-type            | Ckm       | 0.19             | up                    | 0.0057  |

1 Log2 Fold Change by Category (Purple Carrots/Control); 2 p value of the t-test less than 5% Benjamini–Hochberg threshold (0.0058).
quality control process of protein folding in ER through recognizing, retaining and refolding the
as well. Canx family catalyzes the formation of disulphide bonds, thereby regulating protein folding
as major molecular chaperones [20]. Both PDI family (Canx, Calr, Hspa1b, Hsp90b1,
Pdia3, Pdia4, Pdia6) and heat shock protein family A member 5 (Hsp90b1), PDI family (Pdia 3, 4 & 6) and heat shock protein 90, beta, member 1 (Hspa5), PDI family catalyze protein folding while the PDI family catalyzes the formation of disulphide bonds, thereby regulating regulates protein folding as well.

Table 4. Enriched gene ontology biological process terms and KEGG pathways in the list of differentially expressed proteins with Purple Potatoes in adipose tissue that are involved in protein folding, lipid metabolism and cholesterol efflux.

| Biological Theme | GO (BP) and KEGG Pathway 1 | Gene Names 2 | p-Value 3 |
|-----------------|----------------------------|--------------|-----------|
| Protein Folding | GO:0006457–protein folding | Uggt1, Canx, Calr, Hspa1b, Hsp90b1, Pdia3, Pdia4, Pdia6 | 1.46 × 10^{-8} |
|                 | mro04141–protein processing in endoplasmic reticulum | Uggt1, Canx, Calr, Galnb, Hspa1b, Hsp90b1, Hspa5, Hgun1, Pdia3, Pdia4, Pdia6, Rpn1, Rpn2 | 7.66 × 10^{-10} |
| Lipid Metabolism | GO:0006633–fatty acid biosynthetic process | Acaca, Apoa1, Apoc1, Apoc2, Fasn | 2.95 × 10^{-3} |
|                 | GO:0008610–lipid biosynthetic process | Hmgcs2, Acaca, Apoa1, Apoc1, Apoc2, Apoe, C3, Fasn, Pck1 | 2.29 × 10^{-3} |
|                 | GO:0016042–lipid catabolic process | Apoa1, Apoa2, Apoc1, Apoc2, Apoe, C3, Pck1 | 2.71 × 10^{-4} |
|                 | GO:0006641–triglyceride metabolic process | Apoa1, Apoa1, Apoc2, Apoc1, C3, Pck1, Pck1 | 2.65 × 10^{-7} |
|                 | GO:0033344–cholesterol efflux | Apoa1, Apoa2, Apoc1, Apoc2, Apoe | 3.68 × 10^{-5} |
|                 | GO:0043691–reverse cholesterol transport | Apoa1, Apoa2, Apoe | 1.34 × 10^{-3} |

1 GO (BP) is Gene Ontology (GO) biological process component (BP) and KEGG pathway is Kyoto Encyclopedia of Genes and Genomes biological pathway; 2 Gene names in bold are upregulated with Purple Potatoes diet while the un-bold names are downregulated with the Purple Potatoes diet in adipose tissue; 3 p-value of the enrichment analyses is significant at Benjamini <0.05.

Table 5. Enriched gene ontology biological process terms and KEGG pathways in the list of differentially expressed proteins with Purple Carrots in adipose tissue that are involved in lipid metabolism and cholesterol efflux.

| Biological Theme | GO (BP) and KEGG Pathway 1 | Gene Names 2 | p-Value 3 |
|-----------------|----------------------------|--------------|-----------|
| Lipid Metabolism | GO:0006633–fatty acid biosynthetic process | Erlin2, Acaca, Anxa2, Apoa4, Apoc2, Apoc3, Fasn, Mgl1 | 9.44 × 10^{-4} |
|                 | GO:0008610–lipid biosynthetic process | Hmgcs2, Erlin2, Ablad5, Acaca, Acal1, Albdha1, Akr1d1, Anxa1, Apoa4, Apoc2, Apoc3, C3, Fasn, Gpat3, Mgl1, Pck1 | 1.14 × 10^{-3} |
|                 | GO:0016042–lipid catabolic process | Ablad5, Acal2, Akr1d1, Apoa2, Apoc2, Apoc3, Apoh, Cps1, Csa1d, Ehhadh, Fabp1, Hadha, Hadhb, Liphe, Mgl1, Pck1, Pkaca | 3.35 × 10^{-8} |
|                 | GO:0009062–fatty acid catabolic process | Acaca2, Ces1d, Ehhadh, Fabp1, Hadha, Hadhb, Liphe | 5.28 × 10^{-4} |
|                 | mro04923–Regulation of lipolysis in adipocytes | Gnas, Ablad5, Liphe, Mgl1, Pck1, Prkaca | 5.01 × 10^{-3} |

1 GO (BP) is Gene Ontology (GO) biological process component (BP) and KEGG pathway is Kyoto Encyclopedia of Genes and Genomes biological pathway; 2 Gene names in bold are upregulated with the Purple Carrots diet while the un-bold names are downregulated with the Purple Carrots diet in adipose tissue; 3 p-value of the enrichment analyses is significant at Benjamini <0.05.

3.1.1. Protein Folding and Endoplasmic Reticulum (ER) Stress

“Protein processing in ER” pathway and “protein folding” biological process were both strongly enriched in the differentially expressed protein list with the PP (Table 4). All the proteins involved in both the pathway and the biological process were upregulated with the PP diet. UDP-glucose glycoprotein glucosyltransferase 1 (Uggt1), calnexin (Canx) and calreticulin (Calr) are involved in quality control process of protein folding in ER through recognizing, retaining and refolding the immaturely folded proteins [19]. Uggt1 recognizes proteins with folding defects, retains them and directs them to Canx/Calr cycle to be refolded properly. Heat shock protein family A member 5 (Hspa5), PDI family (Pdia 3, 4 & 6) and heat shock protein 90, beta, member 1 (Hsp90b1) are also recognized as major molecular chaperones [20]. Both Hspa5 and Hsp90b1 catalyze protein folding while the PDI family catalyzes the formation of disulphide bonds, thereby regulating regulates protein folding as well.
Accumulation of misfolded or unfolded proteins results in ER stress. ER stress response or UPR (unfolded protein response) is known as a common mechanism of the pathogenesis of IR. For instance, UPR recruits and activates a number of stress kinases that eventually impair insulin signaling pathway through inducing serine phosphorylation of IRS1. Moreover activation of the stress kinases promotes proinflammatory cytokines synthesis that also negatively affects insulin signaling [21]. Our finding is consistent with the observation that purple sweet potato color reduced the levels of the ER stress markers, phospho-pancreatic endoplasmic reticulum resident kinase (p-PERK), phospho-eukaryotic translation initiation factor (p-eIF2) and phospho-inositol-requiring 1 (p-IRE1) in the livers of mice fed high fat diet and also suppressed the ER induced inflammation by decreasing nuclear factor-κB (NF-κB) nuclear translocation [22].

3.1.2. Lipid Metabolism

Lipid Synthesis

Both “fatty acid biosynthetic” and “lipid biosynthetic” processes were enriched in the differentially expressed protein list with both the PP and PC diets (Tables 4 and 5). Among the proteins involved in these BP GO terms are Acetyl-CoA carboxylase alpha (Acaca) and fatty acid synthase (Fasn) that were downregulated with both diets. This indicated that de novo fatty acid synthesis was probably downregulated. ER lipid raft associated 2 (Erlin2) was upregulated with the PC diet. This can be another sign of a decreased de novo fatty acid synthesis with the PC diet. Erlin2 depletion was shown to activate SREBP genes and subsequently increasing fatty acid and cholesterol biosynthesis [23]. However, the upregulation of phosphoenolpyruvate carboxykinase 1 (Pck1), with both diets, could be an indication of an increased fatty acid re-esterification, that could be coupled with the increased glyceroneogenesis. In adipose tissue, cytosolic Pck1 is a key enzyme in glyceroneogenesis that involves the synthesis of glycerol 3 phosphate (G-3-P) by decarboxylating amino acids to phosphoenolpyruvate (PEP) that then converts to dihydroxyacetone phosphate (DHAP), a precursor of G-3-p [24]. The synthesized G-3-P is utilized for fatty acid re-esterification and triglyceride (TG) synthesis in white adipose tissue [24]. In fact, over expression of Pck1 in adipose tissue of mice was shown to increase FFA re-esterification, glyceroneogenesis and obesity while decreasing circulating FFA levels and preserving glucose tolerance and whole body insulin sensitivity [25]. Lipid localization to the adipose tissue will probably decrease the lipid accumulation in other tissues (i.e., lipotoxicity). Intracellular accumulation of lipid intermediates like DAG and ceramides are known to interrupt insulin signaling [26]. Upregulation of Glycerol-3-phosphate acyltransferase 3 (Gpat3), with the PC diet, may be another sign of an increase in fatty acid re-esterification and TG synthesis. Gpat3 is the first enzyme of the TG de novo synthesis pathway. Its increased expression increases TG formation [27]. Both apolipoprotein C1 (A poc1) and apolipoprotein C2 (A poc2) were upregulated with the PP diet whereas A poc2 and A poc3 were upregulated with the PC diet. A poc2 is required for lipoprotein lipase (LPL) activation. The LPL hydrolyzes TG to free fatty acids that are uptaken and deposited to the adipose tissue [28]. However, A poc1 and A poc3 exert the opposite effect of A poc2 on LPL activity [29]. So it is not clear if LPL is activated or inhibited. Hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) downregulation is an indication of a probable decrease in ketogenesis with both diets. Hmgcs2 is a rate limiting enzyme of the ketone bodies biosynthesis [30]. It is a mitochondrial form of the enzyme that catalyzes the condensation of acetyl CoA with acetoacetyl CoA to form HMGCOA [30]. Ketogenesis is induced in long fasting, prolonged exercise and diabetes. Ketone bodies are used as fuels in these cases [30]. Also Hmgcs2 expression increased with starvation and decreased in response to insulin [30]. So it seems that PP fed rats did not need ketone bodies for energy compared to the control group. Or perhaps they just had less acetyl CoA generated from β-oxidation that led to less ketone bodies synthesis.

Lipid Catabolism

The “lipid catabolic” process was enriched with both the PP and the PC while the “fatty acid catabolic” process was enriched with the PC alone and both “TG catabolic” and “TG metabolic” processes were
enriched with the PP alone (Tables 4 and 5). Upregulation of both perilipin1 (Plin1) and carboxylesterase 1D (Ces1d), with both diets, could be indicative of higher lipolysis activity. Both Plin1 and Ces1d are known to be lipolytic proteins. However, Plin1 has a complex role in lipolysis as it exerts opposing effects on basal and catecholamine stimulated lipolysis. Under basal state, Plin1 decreases lipolysis through coating the lipid droplets and preventing the access of the lipolytic enzymes (e.g., hormone sensitive lipase) to the stored lipids. At the same time, Plin1 increases lipolysis by activating adipose triglyceride lipase (ATGL). ATGL hydrolyzes TG releasing FFAs and subsequent TG hydrolysis [31]. Perilipin ablation in mice resulted in higher basal lipolysis and lower stimulated lipolysis. Perilipin null mice were lean but less glucose tolerant [32]. Ces1d was identified as a major lipolytic enzyme in mice [33]. However, it was not confirmed that it has the same effect on the lipolytic activity in human adipose tissue [34]. Furthermore, the concomitant upregulation of Apoc2 and Pck1 may support the idea that the liberated FFAs are not released to the circulation and instead may actually be re-esterified and deposited to the adipose tissue. So generally we can see some evidence of lower FFA release and lower de novo fatty acid synthesis that may explain the improved insulin sensitivity with these diets.

Among the proteins involved in the “fatty acid catabolic” process are Acetyl-CoA acyltransferase 2 (Aca2) and trifunctional protein (Hadha & Hadhb) and they were downregulated with the PC (Table 5). They are the enzymes catalyzing the last steps of the mitochondrial fatty acid β-oxidation. Peroxisomal bifunctional protein (Ehhadh) is also downregulated. Ehhadh is involved in peroxisomal fatty acid β-oxidation as well [35]. The probable decrease in the fatty acid oxidation observed may be due to either the reduced abundance of the newly synthesized fatty acids or directing fatty acids to the re-esterification pathway.

“Regulation of lipolysis in adipocytes” KEGG pathway is also enriched with the PC alone with GNAS complex locus (Gnas), abhydrolase domain containing 5 (Abhd5), hormone sensitive lipase (Lipe), cAMP-activated protein kinase (Prkaca) and Plin1 (Table 5). They were all upregulated with the PC diet. This can be an indication of increased stimulated lipolysis in this group. Under catecholamine stimulation and during fasting, Gnas activates adenylate cyclase with a subsequent increase in cAMP. High levels of cAMP activate Prkaca that phosphorylates both Lipe and Plin1 with a subsequent TG hydrolysis [36]. Plin1 phosphorylation induces a conformational change that gives lipolytic enzymes more access to the adipocytes allowing lipolysis [37]. Abhd5 also positively regulate lipolysis via activating adipose triglyceride lipase (ATGL). ATGL hydrolyzes TG releasing FFAs and DAG [38]. However, also only under the stimulated lipolysis state and Plin1 phosphorylation, Abhd5 gets released from its binding with Plin1 which allows its action on ATGL [31]. During fasting or exercise, the liberated free fatty acids are needed and directed to other tissues to be oxidized for energy. Also Plin1 upregulation may indicate less basal lipolysis. Higher basal lipolysis is suggested to be the cause of IR in Plin1 null mice with low stimulated lipolysis [32]. However, more studies on differentiating the role of stimulated lipolysis versus the role of basal lipolysis in IR are needed.

3.1.3. Cholesterol Efflux/Reverse Cholesterol Transport (RCT)

Both “cholesterol efflux” and “RCT” processes are enriched in the differentially expressed proteins list with the PP (Table 4). “Cholesterol efflux” process is also enriched with the PC (Table 5). Apolipoprotein A1 (Apoa1), Apoa2, Apoc1 and Apoc2 were upregulated while apolipoprotein E (Apoe) was downregulated with the PP diet. Apoa2, Apoa4, Apoc2, Apoc3 and caveolin1 (Cav1) are all upregulated with the PC diet as well. Since Apoa1 and Apoa2 are the most abundant apolipoproteins in high density lipoprotein cholesterol containing particles (HDLc) [28], perhaps the higher protein abundance is simply an indication of overall higher HDLc levels with the PP compared to the control diet. As reported previously, the PP group was more insulin sensitive than the control group; it would not be surprising to see an associated improved lipid profile (i.e., higher HDLc). The association of dyslipidemia with IR is thought to be due to the high VLDL hepatic secretion and the high postprandial chylomicron levels coupled with the exchange
of cholesterol esters from HDLc with TG from TG-rich lipoproteins. This leaves a more hydrolysis and
dissociation prone TG-rich HDL particle, and thus reduces the number of HDL particles [39]. Apoa1
transcription was shown to be modulated by dietary and hormonal factors [40]. Increased human Apoa1
expression in transgenic mice increases HDLc levels and inhibits atherosclerosis [40]. At this point, it is
not clear if the high Apoa1 and Apoa2 are the result of higher insulin sensitivity and higher HDLc with the
PP diet, or due to a direct effect of the PP on the expression of Apoa1 and Apoa2. Furthermore, since Apoe
is typically found on TG-rich lipoproteins (chylomicrons, IDL, VLDL) [28], its decreased expression may
be just a reflection of lower levels of these lipoproteins with the PP diet.

Apoa1 has a major role in cholesterol efflux (i.e., cholesterol acceptor) and is also a main lecithin
cholesterol acyl transferase (LACT) activator that catalyzes cholesterol esterification and promotes
more cholesterol uptake by HDL particles [28]. However, Apoa2, Apoa4, Apoc2, Apoc3 and Cav1 were
all shown to promote cholesterol efflux in vitro [41,42]. This strongly suggests that cholesterol efflux is
enhanced with both diets. Cholesterol efflux is the first step of RCT that involves the removal of the
excess cholesterol from the tissues and delivering it back to the liver for excretion [28].

Cholesterol efflux capacity was progressively reduced in patients with MetS with increasing
number of MetS risk factors [43]. It also was negatively correlated with fasting blood glucose and
systolic blood pressure [43]. Efflux capacity is inversely associated with the risk of coronary heart
disease (CHD) [44]. Although the capacity is positively correlated with the Apoa1 concentration, it is
the capacity, rather than the concentration, that is suggested to be the accurate predictor of CHD [44].

Taken together, these data suggest that decreased de novo lipogenesis, a decrease in basal lipolysis,
increased fatty acid re-esterification, reduced ER stress (with PP alone), and probably increased
cholesterol efflux in adipose tissue, each contributes to the mechanisms responsible for improving
MetS pathologies (insulin sensitivity and hypertension), with PP and PC feeding (Figure 1).

![Figure 1. Suggested mechanisms of action of purple potatoes and purple carrots on Metabolic Syndrome pathologies in liver and adipose tissue. FFA: free fatty acids, WAT: white adipose tissue.](image)

### 3.2. Liver Protein Expression

A total of 941 proteins were identified in the livers of rats fed the PP, the PC and the control diets
(Supplemental Table S3) of which 69 and 62 proteins were differentially expressed with the PP and the
PC respectively. Thirty-seven proteins were downregulated and 32 proteins were upregulated with
the PP diet (Table 6) whereas 29 proteins were downregulated and 33 proteins were upregulated with
the PC diet (Table 7). A total of 26 KEGG pathways and 134 biological processes were enriched in the
proteins list with the PP diet while 20 KEGG pathways and 130 biological processes were enriched
with the PC diet (at Benjamini $p$ value < 0.05) (Supplemental Tables S4 and S6). Some of the enriched
pathways and processes observed were involved in lipid metabolism, carbohydrate metabolism and
oxidative stress (Tables 8 and 9).
Table 6. Differentially expressed proteins with Purple Potatoes diet in liver.

| Differentially Expressed Proteins                                      | Gene Name | Log2 Fold Change | Up- or Down-Regulated | p Value  |
|------------------------------------------------------------------------|-----------|-----------------|-----------------------|----------|
| Carbamoyl-phosphate synthase [ammonia], mitochondrial                  | Cps1      | 0.17            | up                    | 0.0001   |
| Fatty acid-binding protein, liver                                      | Fabp1     | 0.27            | up                    | 0.0001   |
| Long-chain-fatty-acid-CoA ligase 1                                     | Acs1      | 0.1             | up                    | 0.0001   |
| Bucs1 protein                                                          | Acsm1     | 0.19            | up                    | 0.0001   |
| 3-alpha-hydroxysteroid dehydrogenase                                  | Akr1c9    | 0.17            | up                    | 0.0001   |
| Aldh4a1 protein (Fragment)                                             | Aldh4a1   | 0.13            | up                    | 0.0001   |
| Alpha-aminoacidic semialdehyde dehydrogenase                          | Aldh7a1   | 0.17            | up                    | 0.0001   |
| Cystathionine gamma-lyase                                              | Cth       | 0.2             | up                    | 0.0001   |
| Microsomal triglyceride transfer protein                               | Mttp      | 0.15            | up                    | 0.0001   |
| Long-chain-fatty-acid-CoA ligase 5                                     | Acs1      | 0.24            | up                    | 0.0001   |
| Bile acyl-CoA synthetase                                               | Slc27a5   | 0.22            | up                    | 0.0001   |
| Alcohol sulotransferase A                                              | St2a2     | 0.43            | up                    | 0.0001   |
| Aldose reductase-related protein 1                                     | Akr1b7    | 1.41            | up                    | 0.0001   |
| Fatty acid synthase                                                    | Fasn      | −0.18           | down                  | 0.0001   |
| Pyruvate carboxylase, mitochondrial                                    | Pc        | −0.09           | down                  | 0.0001   |
| Serum albumin                                                          | Alb       | −0.13           | down                  | 0.0001   |
| Triokinase/FMN cyclase                                                 | Tkfc      | −0.14           | down                  | 0.0001   |
| Transketolase                                                          | Tkt       | −0.13           | down                  | 0.0001   |
| ATP-citrate synthase                                                   | Acly      | −0.27           | down                  | 0.0001   |
| Sototransferrin                                                        | Tf        | −0.24           | down                  | 0.0001   |
| Pyruvate kinase                                                        | Pkk1      | −0.18           | down                  | 0.0001   |
| Selenium-binding protein 1                                             | Selenbp1  | −0.14           | down                  | 0.0001   |
| Glucose-6-phosphate isomerase                                          | Gpi       | −0.18           | down                  | 0.0001   |
| Purine nucleoside phosphorylase                                       | Pnp       | −0.12           | down                  | 0.0001   |
| Malate dehydrogenase, mitochondrial                                    | Mdh2      | −0.22           | down                  | 0.0001   |
| Keratin, type II cytoskeletal 8                                        | Krt8      | −0.2            | down                  | 0.0001   |
| Glycerol kinase                                                        | Gk        | −0.16           | down                  | 0.0001   |
| Cytochrome P450 2C11                                                    | Cyp2c11   | −0.37           | down                  | 0.0001   |
| Keratin, type I cytoskeletal 18                                        | Krt18     | −0.23           | down                  | 0.0001   |
| Phosphate carrier protein, mitochondrial                               | Slc25a3   | −0.2            | down                  | 0.0001   |
| Isoform 2 of Fibrinogen beta chain                                    | Fgb       | 0.27            | up                    | 0.0001   |
| Acyl-coenzyme A synthetase ACSM5, mitochondrial                        | Acsn5     | −0.35           | down                  | 0.0001   |
| Farnesyl pyrophosphate synthase 1                                      | Fdps      | 0.21            | up                    | 0.0001   |
| Protein disulfide-isomerase                                            | P4hib     | 0.1             | up                    | 0.0002   |
Table 6. Cont.

| Differentially Expressed Proteins | Gene Name | Log2 Fold Change | Up- or Down-Regulated | p Value |
|-----------------------------------|-----------|------------------|-----------------------|---------|
| Choline dehydrogenase, mitochondrial | Chdh     | −0.13            | down                  | 0.0002  |
| Carboxylesterase 1D                | Ces1d    | 0.36             | up                    | 0.0002  |
| Malate dehydrogenase, cytoplasmic  | Malh1    | 0.15             | up                    | 0.0003  |
| Malic enzyme                       | Mel      | −0.15            | down                  | 0.0003  |
| Glutathione peroxidase             | Gpx1     | 0.17             | up                    | 0.0003  |
| Afatoxin B1 aldehyde reductase member 3 | Akr7a3 | −0.26           | down                  | 0.0004  |
| Lactamase, beta                   | Lactb    | −0.14            | down                  | 0.0004  |
| Alpha-ami-noacidic semialdehyde synthase, mitochondrial | Asa | 0.22            | up                    | 0.0005  |
| Perilipin 2                       | Plin2    | −0.41            | down                  | 0.0005  |
| Acyl-coenzyme A oxidase           | Acox3    | 0.09             | up                    | 0.0005  |
| Kynurenine/alpha-ami-noacidip aminotransferase, mitochondrial | Aadat   | 0.18             | up                    | 0.0005  |
| Dihydrolipopolylysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial | Dlat | −0.17          | down                  | 0.0006  |
| Carboxylic ester hydrolase (Fragment) | Ces2e | 0.49            | up                    | 0.0009  |
| Cytochrome P450 2B3              | Cup2b3   | 0.18             | up                    | 0.0009  |
| Estrogen sulfotransferase, isoform 3 | Ste | −0.46          | down                  | 0.001   |
| Glucose-6-phosphate 1-dehydrogenase | G6pdx | 0.35            | up                    | 0.001   |
| Alcohol dehydrogenase 1          | Adh1     | 0.08             | up                    | 0.0012  |
| Isocitrate dehydrogenase [NADP] cytoplasmic | Idh1 | 0.09            | up                    | 0.0012  |
| Glutathione S-transferase alpha-4 | Gst4d    | 0.13             | up                    | 0.0012  |
| Myosin, heavy polypeptide 9, non-muscle | Mdy9 | −0.1          | down                  | 0.0012  |
| Protein deglycase Df-1            | Park7    | −0.26            | down                  | 0.0012  |
| Transgelin-2                     | Tgln2    | −0.21            | down                  | 0.0013  |
| Phosphoenolpyruvate carboxykinase, cytosolic [GTP] | Pck1 | 0.11            | up                    | 0.0014  |
| Long-chain specific acyl-CoA dehydrogenase, mitochondrial | Acadl | −0.1          | down                  | 0.0014  |
| Voltage-dependent anion-selective channel protein 3 | Vdac3 | −0.27          | down                  | 0.0017  |
| Alpha-1-macroglobulin             | A1m      | 0.14             | up                    | 0.0018  |
| Afatoxin B1 aldehyde reductase member 2 | Akr7as2 | −0.15         | down                  | 0.0019  |
| Fructose-bisphosphate aldolase    | Aldob    | −0.11            | down                  | 0.0021  |
| Epoxide hydrolase 1               | Epox1    | −0.11            | down                  | 0.0021  |
| UDP-glucuronosyltransferase 2B2   | Ugt1b2   | 0.17             | up                    | 0.0023  |
| 3 beta-hydroxy steroid dehydrogenase type 5 | Hsd3b5 | −0.24          | down                  | 0.0024  |
| 3-hydroxyisobutyryl-CoA hydrolase, mitochondrial | Hibch | −0.16          | down                  | 0.0027  |
| Cytosol aminopeptidase            | Lap3     | −0.08            | down                  | 0.0028  |
| UDP-glucuronosyltransferase 2B17 OS | Ugt2b17 | 0.27          | up                    | 0.0033  |
| Biliverdin reductase A            | Blvra    | −0.15            | down                  | 0.0033  |

1 Log2 Fold Change by Category (Purple Potatoes/Control); 2 p value of the t-test less than 5% Benjamini–Hochberg threshold (0.0037).
Table 7. Differentially expressed proteins with the Purple Carrots diet in liver.

| Differentially Expressed Proteins | Gene Name | Log2 Fold Change $^1$ | Up- or Down-Regulated | $p$ Value $^2$ |
|-----------------------------------|-----------|-----------------------|------------------------|---------------|
| Carbamoyl-phosphate synthase [ammonia], mitochondrial | Cps1 | 0.05 | up | 0.0001 |
| Cytosolic 10-formyltetrahydrofolate dehydrogenase | Aldh1I1 | 0.14 | up | 0.0001 |
| Catalase | Cat | 0.15 | up | 0.0001 |
| Cytochrome P450 2C7 | Cyp2c7 | 0.29 | up | 0.0001 |
| Alcohol dehydrogenase 1 | Adh1 | 0.14 | up | 0.0001 |
| Alpha-1-macroglobulin | A1m | 0.14 | up | 0.0001 |
| Epoxide hydrolase 1 | Ephx1 | 0.21 | up | 0.0001 |
| Cystathionine gamma-lyase | Cth | 0.19 | up | 0.0001 |
| 4-hydroxyphenylpyruvate dioxygenase | Hpd | 0.25 | up | 0.0001 |
| Glutathione S-transferase | Gsta5 | 0.46 | up | 0.0001 |
| Protein Sar1a | Sar1a | 0.18 | up | 0.0001 |
| Aflatoxin B1 aldehyde reductase member 3 | Akr7a3 | 0.45 | up | 0.0001 |
| Histidine ammonia-lyase | Hid | 0.35 | up | 0.0001 |
| Carboxylesterase 1D | Ces1d | 0.54 | up | 0.0001 |
| Fatty acid synthase | Fasn | −0.13 | down | 0.0001 |
| Aldehyde dehydrogenase, mitochondrial | Aldh2 | −0.25 | down | 0.0001 |
| 3-ketoacyl-CoA thiolase, mitochondrial | Acac2 | −0.27 | down | 0.0001 |
| 60 kDa heat shock protein, mitochondrial | Hspd1 | −0.07 | down | 0.0001 |
| Transketolase | Tkt | −0.25 | down | 0.0001 |
| ATP-citrate synthase | Acyl | −0.3 | down | 0.0001 |
| Malate dehydrogenase, mitochondrial | Mdh2 | −0.22 | down | 0.0001 |
| Keratin, type II cytoskeletal 8 | Krt8 | −0.13 | down | 0.0001 |
| Sorbitol dehydrogenase | Sord | −0.14 | down | 0.0001 |
| Aldehyde dehydrogenase X, mitochondrial | Aldh1b1 | −0.46 | down | 0.0001 |
| Protein LOC679794 | LOC679794 | −0.33 | down | 0.0001 |
| UDP-glucuronosyltransferase 2B2 | Ugt2b | 0.18 | up | 0.0002 |
| Hemoglobin subunit beta-1 | Hbb | −0.24 | down | 0.0002 |
| Pyruvate kinase | Pklr | −0.12 | down | 0.0002 |
| Protein Ugp2 | Ugp2 | 0.25 | up | 0.0002 |
| Isoform 2 of Fibrinogen beta chain | Fgb | 0.21 | up | 0.0002 |
| UDP-glucuronosyltransferase 2B15 | Ugt2b15 | 0.13 | up | 0.0003 |
| Alpha-aminoadipic semialdehyde synthase, mitochondrial | Aass | 0.17 | up | 0.0004 |
| Cytochrome P450 2C23 | Cyp2c23 | 0.2 | up | 0.0004 |
| Argininosuccinate synthase | Ass1 | 0.11 | up | 0.0004 |
### Table 7. Cont.

| Differentially Expressed Proteins | Gene Name | Log2 Fold Change | Up- or Down-Regulated | p Value |
|-----------------------------------|-----------|------------------|------------------------|---------|
| Pyruvate dehydrogenase E1 component subunit alpha | Pdhα1 | −0.22 | down | 0.0004 |
| Keratin, type I cytoskeletal 18 | Krt18 | −0.17 | down | 0.0006 |
| 3-oxo-5-beta-steroid 4-dehydrogenase | Akr1d1 | −0.08 | down | 0.0006 |
| 3-alpha-hydroxysteroid dehydrogenase | Akr1c9 | 0.1 | up | 0.0007 |
| Perilipin 2 | Plin2 | −0.31 | down | 0.0007 |
| Hemoglobin subunit alpha-1/2 | Hba1 | −0.22 | down | 0.0007 |
| Long-chain specific acyl-CoA dehydrogenase, mitochondrial | Acadl | −0.1 | down | 0.0008 |
| Carnitine O-palmitoyltransferase 1, liver isoform | Cpt1a | −0.22 | down | 0.0009 |
| L-gulonolactone oxidase | Gulo | −0.17 | down | 0.0009 |
| Retinol dehydrogenase 7 | Rdh7 | 0.15 | up | 0.0010 |
| Protein deglycase DJ-1 | Park7 | −0.17 | down | 0.0010 |
| Peroxisomal multifunctional enzyme type 2 | Hsd17b4 | 0.07 | up | 0.0015 |
| 60S ribosomal protein L14 | Rpl14 | −0.17 | down | 0.0015 |
| Glutathione S-transferase | Gsta2 | 0.53 | up | 0.0017 |
| Malate dehydrogenase, cytoplasmic | Mdh1 | 0.13 | up | 0.0017 |
| Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial | Dhkd1 | 0.12 | up | 0.0017 |
| 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Mitochondrial) | Hmgcs2 | −0.07 | down | 0.0017 |
| Pterin-4-alpha-carbinolamine dehydratase | Pchd1 | −0.2 | down | 0.0017 |
| Heat shock cognate 71 kDa protein | Hspa8 | −0.06 | down | 0.0018 |
| Non-specific lipid-transfer protein | Sep2 | −0.15 | down | 0.0020 |
| Carbonic anhydrase 3 | Ca3 | 0.48 | up | 0.0022 |
| Protein LOC100911833 | LOC297568 | 0.14 | up | 0.0023 |
| Cytochrome P450 2A2 | Cyp2a2 | 0.38 | up | 0.0023 |
| Cullin-associated NEDD8-disassociated protein 1 | Cand1 | −0.2 | down | 0.0023 |
| Eukaryotic translation elongation factor 1 beta 2 | Eef1b2 | −0.16 | down | 0.0023 |
| Ectonucleoside triphosphate diphosphohydrolase 5 | Entpd5 | 0.17 | up | 0.0027 |
| Glutathione S-transferase alpha-5 | Gsta5 | 0.33 | up | 0.0027 |
| Formimidoyltransferase-cycloeaminase | Ftdc | 0.06 | up | 0.0033 |

1 Log2 Fold Change by Category (Purple Carrots/Control); 2 p value of the t-test less than 5% Benjamini–Hochberg threshold (0.00336).
Table 8. Enriched gene ontology biological process terms and KEGG pathways in the list of differentially expressed proteins with Purple Potatoes in liver that are involved in lipid metabolism and carbohydrate metabolism.

| Biological Theme       | GO (BP) and KEGG Pathway 1 | Gene Names 2                | p-Value 3               |
|------------------------|-----------------------------|-----------------------------|-------------------------|
| Lipid Metabolism       | GO:000633–fatty acid biosynthetic process | Acly, Acd, Acen1, Acsm5, Fasn | 1.58 × 10⁻³              |
|                        | GO:0008610–lipid biosynthetic process | Hsd3b5, Acly, Acd1, Acsl1, Acsl5, Acsm1, Acsm5, Fdps, Fasn, G6pd, Idh1, Pck1, P, Slc27a5 | 6.00 × 10⁻⁸              |
|                        | GO:0016042–lipid catabolic process | Hltc, Acd, Acsl5, Cps1, Ces1d, Fabp1, Idh1 | 7.64 × 10⁻⁵              |
|                        | GO:000635–fatty acid beta-oxidation | Hltc, Acd, Acsl5, Cps1, Ces1d, Fabp1 | 1.32 × 10⁻⁴              |
| Carbohydrate Metabolism| GO:0016052–carbohydrate catabolic process | Aldol, Cps1, Gpi, Gkp, Pkh3 | 1.42 × 10⁻³              |
|                        | rno001030: Pentose phosphate pathway | Aldol, G6pd, Gpi, Tk1 | 1.19 × 10⁻³              |

1 GO (BP) is Gene Ontology (GO) biological process component (BP) and KEGG pathway is Kyoto Encyclopedia of Genes and Genomes biological pathway; 2 Gene names in bold are upregulated with Purple Potatoes diet while the un-bold names are downregulated with the Purple Potatoes diet in liver; 3 p-value of the enrichment analyses is significant at Benjamini < 0.05.

Table 9. Enriched gene ontology biological process terms and KEGG pathways in the list of differentially expressed proteins with Purple Carrots in liver that are involved in lipid metabolism, carbohydrate metabolism and oxidative stress.

| Biological Theme       | GO (BP) and KEGG Pathway 1 | Gene Names 2                | p-Value 3               |
|------------------------|-----------------------------|-----------------------------|-------------------------|
| Lipid Metabolism       | GO:0009062–fatty acid catabolic process | Acsl2, Acd1, Ces1d, Cpt1a, Hsd17b4 | 2.46 × 10⁻⁴              |
|                        | rno001053: Acyl-CoA biosynthetic process | Acly, Fasn, Pldh1, Pldh1n1 | 1.85 × 10⁻⁴              |
|                        | rno00120: Primary bile acid biosynthesis | Akr1d1, Hsd17b4, Scy2 | 4.77 × 10⁻³              |
| Carbohydrate Metabolism| GO:0005975–carbohydrate metabolic process | Ugp2, Cps1, Cpt1a, Dhk1d3, Entpd5, Mdh1, Mdh2, Pldh1, Pkh3, Srd5 | 8.83 × 10⁻⁴              |
| Oxidative Stress       | GO:0006979–response to oxidative stress | Park7, Car3, Cat, Hsp70, Hspa8, Hspd1, Hbb, Hba1 | 1.60 × 10⁻³              |
|                        | rno0042744: Hydrogen peroxide catabolic process | Cat, Hbb, Hba1 | 2.55 × 10⁻³              |

1 GO (BP) is Gene Ontology (GO) biological process component (BP) and KEGG pathway is Kyoto Encyclopedia of Genes and Genomes biological pathway; 2 Gene names in bold are upregulated with the Purple Carrots diet while the un-bold names are downregulated with the Purple Carrots diet in liver; 3 p-value of the enrichment analyses is significant at Benjamini < 0.05.

3.2.1. Lipid Metabolism

Lipid Synthesis

Both “fatty acid biosynthetic” and “lipid biosynthetic” processes are enriched in the list of the differentially expressed proteins with the PP while “acyl CoA biosynthetic” process was enriched with the PC diet (Tables 8 and 9). Downregulation of Fasn, pyruvate carboxylase (Pc) and ATP citrate lyase (Acly) with the PP as well as downregulation of Acly, Fasn and pyruvate dehydrogenase alpha 1 (Pdha1) with the PC likely indicate a decrease in de novo fatty acid synthesis with both diets. Pc catalyzes the conversion of pyruvate to oxaloacetate that condenses with acetyl CoA to produce citrate. In the cytoplasm, Acly converts citrate back to acetyl CoA which is then used in fatty acid synthesis [45]. In db/db mice, ablation of hepatic citrate lyase prevents de novo lipogenesis and hepatic steatosis and promotes insulin sensitivity in muscle [46]. Pdha1, like Acyl, is an acetyl CoA source.

Farnesyl diphosphate synthase (Fdps) and solute carrier family 27 member 5 (Slc27a5) were both upregulated with the PP (Table 8). Fdps catalyzes the formation of farnesyl pyrophosphate that constitutes a branching point of the isoprenoid pathway that yield both sterol and non-sterol metabolites [47]. Slc27a5 is a bile acyl CoA synthase that is involved in bile acid conjugation and activation before excretion into the bile canaliculi [48]. So, even though the upregulation of Fdps can be a sign of increased de novo cholesterol synthesis, the upregulation of slc27a5 suggests an increased incorporation of the synthesized cholesterol into bile acid biosynthesis with the PP. Bile acid formation
from cholesterol is a main cholesterol excretion route [47]. Primary bile acid synthesis was also enriched with the PC (Table 9). However, even though Hsd17b4 is upregulated, Akr1d1 and sterol carrier protein 2 (Scp2) are downregulated. All three proteins are involved in bile acid biosynthesis [49–51]. So no conclusion on bile acid synthesis can be made with the PC.

Both acyl-CoA synthetase long-chain family member 1 (Acsl1) and acyl-CoA synthetase long-chain family member 5 (Acsl5) are upregulated with the PP. Long chain acyl CoA synthases are a group of enzymes that catalyze the formation of acyl CoAs that can then be directed to either lipid synthesis or oxidation [52]. Acsl1 is suggested to be mainly involved in TG synthesis whereas Acsl5 is suggested to be involved in β-oxidation [52]. However, data from a loss of function in vitro study, observed a role for Acsl5 in directing fatty acids to TG synthesis [53]. In another loss of function study, hepatic Acsl1 was suggested to have a role in both β-oxidation and TG synthesis [54]. Because both pathways may be activated, it would be important to know the relative activation of one pathway over the other (i.e., enzyme activities and/or metabolite levels) to determine whether there would be overall change.

### Lipid Catabolism

“Lipid catabolic” and “fatty acid β-oxidation” processes were enriched in the list of the differentially expressed proteins extracted from the liver tissues of the PP group while the “fatty acid catabolic” process was enriched with that of the PC group (Tables 8 and 9). Fatty acid β-oxidation seems to be downregulated with both diets. Acyl-CoA dehydrogenase, long chain (Acadl) was found to be downregulated with the PP. Also Acadl, Acaa2, and carnitine palmitoyltransferase 1A (Cpt1a) were all downregulated with the PC. Acadl and Acaa2 catalyze the first and the last steps of β-oxidation pathway respectively whereas Cpt1a is the enzyme that is responsible for transporting fatty acids to the mitochondria for oxidation [35]. The probable decrease in the fatty acid oxidation could be due to the observed decrease in the abundance of the fatty acids as a result of reduced de novo lipogenesis. However, the Upregulation of acyl-CoA oxidase 3 (Acox3) and cytosolic isocitrate dehydrogenase (Idh1), with the PP, as well as, the upregulation of d bifunctional protein (Hsd17b4), with the PC, is probably a sign of higher peroxisomal fatty acid β-oxidation in the liver. Acox3 is a rate limiting enzyme in β-oxidation pathway of the peroxisome as it catalyzes the oxidation of methyl branched fatty acyl CoAs and to a lesser extent straight chain fatty acids [35]. Also, cytosolic Idh1 was shown to be necessary for peroxisomal β-oxidation of unsaturated fatty acids in rat liver cells through provision of NADPH [55]. Hsd17b4 is also involved in peroxisomal fatty acid β-oxidation [49].

### 3.2.2. Carbohydrate Metabolism

The “carbohydrate catabolic” process and “pentose phosphate” KEGG pathway were enriched with the PP while “carbohydrate metabolic” process was enriched with the PC (Tables 8 and 9). Glycolysis seems to be decreased with both diets as glucose-6-phosphate isomerase (Gpi), fructose-bisphosphate aldolase B (Aldob) and pyruvate kinase (Pklr), 3 enzymes of the glycolytic pathway [56], and dihydrolipoamide S-acetyltransferase (Dlat) are all downregulated with the PP diet while both Pklr and Pdha1 are downregulated with the PC diet. Dlat is a component of pyruvate dehydrogenase complex that converts pyruvate to acetyl CoA that gets directed to the citric acid cycle or used for de novo lipogenesis.

While glycolysis seems to be decreased, glycogen synthesis pathway proteins (i.e., glycogen synthase) do not seem to be higher in PP livers compared to control liver. However, it does seem that glucose is being directed to the pentose phosphate pathway, as glucose 6 phosphate dehydrogenase (G6pd) is upregulated with the PP. It is true that transketolase (Tkt) is downregulated but it is more involved in the non-oxidative part of the pathway that produces more glycolytic intermediates. The main products of the pentose phosphate pathway are NADPH and ribose 5 phosphate. NADPH is known to be used in fatty acid and cholesterol biosynthesis and in the reduction of oxidized glutathione [57]. Reduced glutathione may confer antioxidant protective effects as it reduces oxidized glutathione peroxidase [58]. It is worth noting that Glutathione peroxidase (Gpx1), the enzyme that reduces H₂O₂ [58], is also among the upregulated proteins in the PP list.
On the PC side, upregulation of UDP-glucose pyrophosphorylase 2 (Ugp2) may be a probable indication of increased glycogen synthesis with the PC. Ugp2 catalyzes the reversible synthesis of UDP glucose which is the immediate precursor of glycogen synthesis [59]. Sorbitol dehydrogenase (Sord) is also downregulated with the PC. Sord is the second enzyme of the polyol pathway where glucose is converted to sorbitol then fructose by the action of Sord. However, its catalytic action is suggested to contribute to oxidative stress by producing NADH that produces ROS by the action of NADH oxidase [60].

3.2.3. Oxidative Stress

“Response to oxidative stress” and “hydrogen peroxide catabolic” biological processes (Table 9) are enriched with the PC alone. Upregulation of catalase (Cat), enzyme catalyzing the conversion of H$_2$O$_2$ to water and O$_2$ [61], can be a sign of antioxidant protective effects. Also, downregulation of both hemoglobin subunit beta and hemoglobin alpha 1 (Hbb and Hba1) may be a sign of less oxidative stress with PC group. The expression of both proteins was higher in fatty liver disease that was suggested to be due to the associated higher oxidative stress [62]. Similarly, heat shock protein family A (Hsp70) and heat shock protein family D member 1 (Hspd1) expression and phosphorylation respectively were induced in response to oxidative stress [63,64]. Parkinsonism associated deglycase (Park7) is a redox sensitive protein that was shown to be upregulated in vitro under oxidative stress conditions [65].

So downregulation of Hspa8, Hspd1 and Park7 may also be a sign of less oxidative stress with PC. Oxidative stress is an established player in promoting IR [66] and hypertension [67]. In fact, oxidative stress may be one of the links between fat accumulation in the liver and IR [68]. Oxidative stress interrupts insulin signaling through activating stress kinases and serine-phosphorylating IRS1 [68]. Furthermore, ROS induces endothelial dysfunction as one way of developing hypertension [67]. Oxidative stress is seen as a common pathological mechanism between fatty liver and CVD [69].

These findings are in agreement with multiple studies that observed antioxidative damage properties of purple vegetables. For instance, consumption of purple potatoes significantly reduced the concentrations of 8-hydroxydeoxyguanosine, a marker of oxidative stress induced DNA damage in men [70]. Purple carrot juice also decreased plasma oxidative stress markers such as malondialdehyde levels [71]. In vitro purple vegetable extracts were able to increase the activity of several antioxidant enzymes such as CAT, GPx and superoxide dismutase [72].

Taken together, these data suggest that a decrease in hepatic de novo lipogenesis, a probable increase in the peroxisomal fatty acid oxidation and a decrease in the fatty acid delivery to the liver from the adipose tissue, each contributes to the mechanisms responsible for improving MetS pathologies with PP and PC feeding (Figure 1). All of the aforementioned signs are mechanisms involved in hepatic lipid accumulation [73]. A decrease in hepatic de novo lipogenesis improves hepatic insulin sensitivity [73]. Lipid metabolites, such as DAG, induce IR in the liver by activating protein kinase C and serine-phosphorylating IRS1 [73]. Reducing oxidative damage may also be contributing to the positive effects of these vegetables on MetS pathologies in the liver (Figure 1).

Some of the current study findings are consistent with other proteomic studies that looked at the adipose proteomic profile changes in response to rosiglitazone [74], resveratrol [75], and caloric restriction [76]. The modulated proteins were involved in lipid metabolism such as perillpin with rosiglitazone [74] and APOA1, fatty acid binding proteins and aldoketoreductases with caloric restriction [76] and oxidative stress such as catalase and superoxide mutase with rosiglitazone [74] and peroxiredoxin and heat shock protein 70 with resveratrol [75]. Heat shock proteins involved in protein folding were also modulated with rosiglitazone [74].

4. Conclusions

There are some obvious similarities between the two purple vegetables in the enriched biological processes, the involved proteins and finally in the main suggested mechanisms of action in the liver and adipose tissue. Overall, we provided a molecular basis of the metabolic benefits of these vegetables that
substantiate the results of our previous study on the metabolic phenotypic parameters. Interestingly, there appear to be many more regulated target proteins in the adipose tissue compared to the liver. This is somewhat surprising given the assumed central role for liver in handling macronutrients and phytochemicals. It does however, point to the now very much appreciated role of adipose tissue in regulating metabolism. No longer do we consider adipose as a benign fat depot but rather a pivotal regulator of the entire metabolic phenotype.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/10/4/456/s1, Table S1: The list of identified proteins in the adipose tissue, Table S2: Enriched gene ontology biological process terms and pathways in the list of the differentially expressed proteins with purple potatoes diet in adipose tissue, Table S3: The list of identified proteins in liver, Table S4: Enriched gene ontology biological process terms in the list of the differentially expressed proteins with purple potatoes diet in liver, Table S5: Enriched gene ontology biological process terms and pathways in the list of the differentially expressed proteins with the purple carrots diet in adipose tissue, Table S6: Enriched gene ontology biological process terms in the list of the differentially expressed proteins with the purple carrots diet in liver.

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