Intake of Watermelon and Watermelon Byproducts Male Mice Fed a Western-style Obesogenic Diet Alters Hepatic Gene Expression Patterns as Determined by RNA Sequencing

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Abstract:

Background

Consumption of watermelon has been associated with beneficial effects on metabolism including reductions in systolic blood pressure, improved fasting blood glucose levels, and changes in hepatic metabolite accumulation.

Objective

In the present study, we investigated the impact of consumption of watermelon flesh (WF), rind (WR), and skin (WS) on hepatic gene expression patterns in an obesogenic mouse model.

Methods

Following a ten-week feeding trial during which C57BL/6J mice were provided either low-fat (LF) diet, high-fat (HF) diet, or high-fat plus watermelon skin (WS), watermelon rind (WR),
or watermelon flesh (WF), hepatic RNA was isolated and RNA sequencing was performed. Bioinformatic approaches were used to determine changes in canonical pathways and gene expression levels for lipid- and xenobiotic-regulating nuclear hormone receptors and other related transcription factors including AhR, CAR, FXR, PPARα, PPARγ, LXR, PXR, and Nrf2.

Results

There were 9,394 genes that had unchanged expression levels between all 5 diet groups, and 247, 58, and 34 genes uniquely expressed in the WF, WR, and WS groups, respectively. Relative levels of mRNAs regulated by AhR, CAR, and PPARα were upregulated in mice consuming WF compared to HF fed mice, whereas mRNAs regulated mainly by CAR were upregulated in mice consuming WR and WS compared to HF.

Conclusions

At modest levels of intake reflective of typical human consumption, mice consuming WF, WS, and WR exhibited hepatic gene expression profiles altered from HF. Several of these changes involve genes regulated by ligand-responsive transcription factors implicated in xenobiotic and lipid metabolism, suggesting that modulation of these transcription factors occurred in response to consumption of watermelon skin, rind, and flesh. Some of these changes are likely due to nuclear hormone receptor-mediated changes involved in lipid and xenobiotic metabolism.

Keywords: watermelon, RNA Sequencing, obesity, diabetes, metabolic syndrome, mice, nuclear hormone receptors.
AhR – Aryl hydrocarbon Receptor

CAR – Constitutive Androstane Receptor

Crp – C-reactive protein

eNOS – endothelial nitric oxide synthase

FA – fatty acid

FXR – Farnesyl X Receptor

GO – Gene Ontology

HF – high fat diet

LF – low fat diet

LXR – Liver X Receptor

MetS – Metabolic syndrome

NAFLD – non-alcoholic fatty liver disease

NASH – non-alcoholic steatohepatitis

NEFA – non-esterified fatty acids

NO – nitric oxide

Nrf2 – Nuclear factor erythroid 2-related factor 2

PPARα – Peroxisome Proliferator Activated Receptor alpha

PPARγ – Peroxisome Proliferator Activated Receptor gamma

PXR – Pregnane X Receptor
1 Introduction

Metabolic syndrome (MetS) is a condition that affects a quarter of the global population, and defined as the co-occurrence of metabolic risk factors that includes insulin resistance, hyperinsulinemia, impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, and visceral obesity (1,2), which substantially increases an individual’s risk of developing non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, cardiovascular disease, and cancer (3).

Non-Alcoholic Steatohepatitis (NASH) and NAFLD are characterized by excessive fat accumulation in the liver (4). Eating patterns rich in saturated fats, cholesterol, and simple sugars, such as the standard Western diet, contribute to hepatic lipotoxicity, and play an important role in development and progression of NAFLD and NASH (5). Further evidence suggests that beyond low-carbohydrate and low-fat approaches, inclusion of vitamin C, D, and E, as well as antioxidant compounds from fruits intake may have a protective effect (6,7). Research has shown that the inclusion of fruits or their extracts in combination with a Western-style diet can ameliorate some of the negative metabolic consequence, by decreasing the deposition of fat in liver and modulating the activity of several transcription factors that regulate the metabolism of lipids, such as PPARα and SREBP-1c (7,8).
Watermelon ingestion has also been associated with reductions in systolic blood pressure, potentially through the action of L-citrulline, which increases serum L-arginine once absorbed and metabolized. L-arginine in the blood results in an increase of nitric oxide (NO) via the action of endothelial nitric oxide synthase (eNOS), which induces vascular smooth muscle relaxation (9), and reduces oxidative stress by scavenging or preventing the formation of hydroxy radicals (10). Nitric oxide is involved in blood vessel dilation, as well as reductions in leukocyte adhesion and platelet aggregation. While NO is produced directly from L-arginine, L-citrulline may be a more effective alternative for increasing NO synthesis in vivo. L-arginine is largely metabolized by arginase in the intestinal lumen, while L-citrulline evades enzymatic modification and is easily absorbed (11). Watermelon contains a variety of other phytochemicals that have beneficial effects on human health including dietary fiber, vitamin C, vitamin E, β-carotene, lycopene, and flavonoids (12). Consequently, other benefits of watermelon have recently been studied, including increased satiety and glucose tolerance (13), and decreased LDL and total cholesterol (14).

Some of the nuclear hormone receptors are ligand-dependent transcription factors that regulate gene expression in a variety of physiological pathways including metabolic processes and effecting epigenetic changes to control transcription (15,16). Ligand-activated nuclear hormone receptors may bind dietary compounds, contributing to coordination of nutrient homeostasis and xenobiotic metabolism. Bioactive compounds naturally present in food, such as polyphenols, fatty acids, and carotenoids, are an interesting pool of potential ligands as they have been refined under evolutionary pressures (16). Low fruit and vegetable intake of less than 400 g per day, excluding potatoes and other root vegetables (17), results in minimal intake of bioactive phytochemicals, which appears to be associated with increased adiposity as well as NAFLD and NASH. More recently, the adverse outcome pathway framework has
been used to contextualize the role of receptors as transcription factors in hepatic steatosis (18).

Previously, Becraft et al. (19) evaluated the impact of a high fat diet [45% fat (by energy), 20% kcal sucrose (by energy), and 1% (w/w) cholesterol] plus watermelon flesh (WF), watermelon rind (WR), or watermelon skin (WS) in ten-week-old male C57BL/6J mice (n=12/control group, n=8/supplemented group). These diets improved fasting blood glucose and produced changes in hepatic metabolite accumulation, especially as related to fatty acid (FA) metabolism and inflammation. In the present study, we investigated the effect that consumption of watermelon flesh (WF), rind (WR), and skin (WS) had on the hepatic transcriptome of these mice, providing a more detailed and mechanistic investigation of watermelon’s influence in a obesogenic animal model.

2 Material and methods

2.1 Watermelon preparation

Fresh watermelon (Citrullus lanatus var. lanatus cv. Fascination) grown in Hermiston, OR was prepared as described by Becraft et al. (19). Briefly, watermelon skin was peeled from the outer surface using a common kitchen peeler and the flesh and rind was sliced into 2”x2”x1/8” sections. Watermelon skin was placed on a metal baking tray and dried for approximately 1.5 hours at 80°C in a drying oven. Watermelon rind was placed on a metal baking tray and dried for approximately 2.5 hours at 80°C drying oven. Watermelon flesh was dried for approximately 4 hours in a 74°C food dehydrator (Dehydro TM, National Presto Industries Inc., Eau Claire, WI, USA). The dried fruit was powdered and incorporated into the experimental diets. Watermelon flesh, rind, and skin was 10.6, 6.6, and 9.6 % moisture, 89.4, 93.4, and 90.4 % solids, and 4, 46.2, and 64.5 % of dietary fiber, respectively (19).
2.2 Mouse diet studies

Forty-eight male C57BL/6J mice (Jackson Laboratory) were randomly divided into two control groups (n=12 each) and three experimental groups (n=8 each) at 6 weeks of age. After a 4-week acclimatization, groups were provided with experimental diets for 10 weeks. These Western-style obesogenic mouse diet, also referred to as high-fat (HF) diet contains 45% kcal fat + 20% kcal sucrose + 1% (w/w) cholesterol. There were two control groups: a low-fat (LF) diet (10% kcal fat) and HF diet; and three treatment groups: HF diet supplemented with dried watermelon flesh at 8% total energy (kcal) (WF), watermelon rind (WR) at 2.25% (w/w), or watermelon skin (WS) at 2.25% (w/w) diets (Supplementary Table 1). Diets were macronutrient balanced such that every HF-based diet contained the same total percentage of each macronutrient, but with different fiber and phytochemical content depending on the foods added as previously described by Becraft et al. (19). Watermelon flesh was included in the diets at 8% of total energy, to model human intake of two servings of watermelon per day (i.e., 160 Calories in a 2,000 Calorie daily diet). Watermelon rind and skin powders were included in diets at 2.25% (w/w) to model the use of common dietary fiber supplements.

At the end of the study, mice were fasted for five hours, anesthetized with isoflurane, and euthanized via cardiac puncture followed by cervical dislocation. Liver tissue was collected, flash frozen on dry ice, and stored at -20°C in RNA-later. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Oregon State University and experiments were approved by the Oregon State University Animal Care and Use Committee (Protocol #4455).
2.3 RNA-Seq

2.3.1 RNA extraction, library preparation, and sequencing

Total liver RNA was extracted from liver tissue (~50mg) using Trizol reagent (Invitrogen Life Technologies, Grand Island, NY, USA) following the standard protocol. The quantity and quality of the isolated RNA was analyzed using a Nano Drop 2000 spectrophotometer (Thermo Scientific, United States). The three samples from each group with the highest RIN, λ260/280, and λ260/230 score were used for RNA-seq. Sequencing libraries were generated using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer’s standard protocols. The six constructed mRNA libraries were sequenced on an Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) at Novogene Technology Co., Ltd (Novogene Gene Technology, Beijing, China).

2.3.2 Differential expression analysis

Raw data (raw reads) in fastq format were first processed through in-house perl scripts. In this step, clean data (clean reads) were obtained by removing reads containing adapters added during the sequencing process to allow amplification of genomes, poly-N, and low-quality reads (in which more than 50% of bases had a quality value (Q-score) ≤20) from raw data. All downstream analyses were based on cleaned data. Reads were obtained from Gene Expression Analysis to perform differential expression analysis using the DESeq2 R package (two conditions/groups), while the significant criterion was an adjusted p-value of ≤0.05.

Global gene expression in the WF, WR, and WS groups were compared to the HF group. To identify genes likely to be direct targets of transcription factor-dependent regulation of hepatic receptors in male mice in response to watermelon diets, we utilized genes known to
be regulated by AhR (Aryl hydrocarbon Receptor) (20), CAR (Constitutive Androstan Receptor) (20), PPARα (Peroxisome Proliferator Activated Receptor α) (20), PPARγ (Peroxisome Proliferator Activated Receptor γ) (21), Nrf2 (Nuclear factor erythroid-2 related factor 2) (20), PXR (Pregnane X Receptor) (20,22), LXR (Liver X Receptor) (23,24), and FXR (Farnesyl X Receptor) (25) as described in the literature (Supplementary Table 2). Due to the multiple differences in the two control diets, we choose in this report, not to detail the differences between LF and HF mice.

Canonical pathways were ascertained using the Gene Ontology (GO) database for biological processes. The most significant up- and down-regulated pathways based of expression values can be found in Tables 1-3.

3 Results

Previously, we evaluated the WF, WR, and WS diets in male C57BL/6J mice fed a western-style obesogenic diet. We reported that body weight was significant higher in HF-fed mice than LF-fed, and no difference between watermelon groups and HF-fed was found; energy intake was higher in WS-fed, followed HF- and WR-fed, and LF-fed mice; and energy efficiency was higher in WR- and WS-fed than LF-, HF-, and WF-fed mice. HF-fed mice supplemented with WF, WS, and WS had reduced fasting blood glucose concentrations such that they did not differ from those of the LF- and HF-fed control groups. The WR group showed insulin concentration lower than HF group while WF and WS groups showed no significant difference with HF group. As for the serum biomarker quantification, MCP-1 was significantly lower in WS group than HF group, and no significant differences were observed for resistin concentrations. Metabolomic analysis demonstrated that a set of liver lipid species were changed with consumption of watermelon products. Further, cecal bacteria populations
from WS-fed mice shifted toward LF-fed mice. Thus, when compared to HF-fed mice, obese mice supplemented with each of the three watermelon products showed improved fasting blood glucose, circulating serum insulin concentrations, and/or changes in hepatic metabolite accumulation (19).

In the present work, to characterize the effect of 10 weeks of dietary intervention on global liver gene expression, the transcriptome of these LF, HF, WF, WR, and WS mice were analyzed by RNA sequencing and differential expression analysis.

The RNA-Sequencing data had an error rate of 0.03 %, Q20 > 97 %, and Q30 > 92 % (Supplementary Table 3). A total of 20,349 different transcripts were analyzed (Figure 1). There were 9,394 that had unchanged expression levels in all 5 diet groups (Fig. 1A). There were 78, 34, 247, 58, and 34 genes uniquely expressed in the LF, HF, WF, WR, and WS groups, respectively (Figure 1A and 1B). When the three watermelon groups were compared, there were 9,811 genes that had unchanged expression levels in WF, WR, and WS diets. There were 322, 99, and 62 genes uniquely expressed in the WF, WR, and WS groups, respectively (Figure 1C).

The 20 canonical pathways most impacted by diet (WF-, WR-, and WS-groups compared to the HF-group), both up and down, are shown in Tables 1-3. For all three of the upregulated pathway sets (WF/HF, WR/HF, and WS/HF), it is apparent that many pathways related to lipid metabolism are upregulated. As an example, fatty acid metabolic processes are the most significantly regulated pathway in WF/HF, with lipid homeostasis and lipid localization also in the top 20 (Table 1). For WR/HF, at least 6 of the top 20 canonical pathways relate to lipid metabolism, including steroid, sterol, and cholesterol metabolic processes and homeostasis (Table 2). Circadian regulation and rhythm are the two most regulated pathways for WR/HF. For WS/HF, more than half of the most regulated pathways
directly relate to lipid metabolism, for example, the five most regulated pathways for WS/HF are fatty acid metabolic processes, lipid localization, lipid transport, regulation of lipid localization, and fatty acid biosynthetic processes (Table 3).

For the case of down-regulated canonical pathways, nearly every one of the most regulated pathways for WF/HF are related to purine nucleoside metabolism. Protein folding and response to endoplasmic reticulum stress are also in the top 20 down-regulated pathways. For the case of WR/HF, most of the top 20 pathways relate to down-regulation of the unfolded protein response pathway, with three other pathways related to inflammation and the immune response. For WS/HF, the unfolded protein response is again prominent, with at least eight other pathways related to down-regulation of vasoconstriction.

Lists of the 20 most up- and down-regulated genes for WF, WR, and WS groups compared to HF are shown in Tables 4-6, respectively. Expression ratios for WF/HF include lipid-related genes such as Acot3 (2.96-fold), Abcd2 (2.37-fold), Abcb1a (2.08-fold), Cyp4a10 (1.86-fold), and Cyp2a22 (1.70-fold); and extracellular matrix organization genes such as Col3a1 (1.75-fold), Colla2 (1.74-fold), and Colla1 (1.73-fold). The WR/HF expression ratios resulted in an upregulation of lipid metabolism genes such as Ptgds (2.31-fold), Abcd2 (1.56-fold), and Tyrp1 (1.46-fold); extracellular matrix organization genes such as Fbfl (1.69-fold); rhythmic process and circadian rhythm regulation genes such as Per3 (1.52-fold) and Ciart (1.24-fold); and carbohydrate and glucose homeostasis signaling genes such as Igfbp5 (1.54-fold). Expression ratios for WS/HF lipid metabolism genes such as Acot3 (1.84-fold), Lipg (1.19-fold), Acot2 (1.13-fold), Pcsk4 (1.12-fold), and Fads3 (1.03-fold); extracellular matrix organization genes Colla1 (1.28-fold) and Colla2 (1.24-fold); and xenobiotic metabolism and transport-related genes including Gstm2 (1.35-fold) and Abcb1a (1.14-fold).
Regarding most down-regulated genes, all three watermelon treated groups had a varied set of mRNAs related to various metabolic pathways. WF/HF mRNAs identified include several genes related to sterol and xenobiotic metabolism. For WR/HF, some of the down-regulated genes indicate a reduction in hepatic inflammation as \textit{Cxcl1} (-1.44-fold), \textit{Inhbb} (-1.27-fold), and \textit{S100a8} (-1.04-fold) (Table 5). With WS/HF, the most down-regulated genes are \textit{Lars2} (-2.02-fold), \textit{Dhrs9} (-1.42-fold), and \textit{Scara5} (-1.37-fold) (Table 6).

To identify genes that are likely to be direct targets of ligand-regulated transcription factors, we produced lists of genes known to be regulated by the AhR, CAR, FXR, LXR, Nrf2, PPAR\textalpha{}, PPAR\gamma{}, and PXR receptors. Lists of mRNAs that are significantly regulated (P<0.05) or showing a trend to significance (0.10>P>0.05) are in sets for WF/HF (Table 7), WR/HF (Table 8), and WS/HF (Table 9). Regarding WF/HF (Table 7), there are 48 mRNAs listed, with 22 of them being known to be regulated by PPAR\textalpha{}, 13 by CAR, and 10 by AhR. For the WR/HF comparison (Table 8), there were fewer mRNAs associated with the list of transcription factors, with only PPAR\textalpha{} having 15 mRNAs. For the case of WS/HF (Table 9), there were 22 mRNAs associated with PPAR\textalpha{}, 10 associated with CAR, and 7 with PXR.

Finally, to focus on the transcription of cytokine genes indicative of inflammation, we show four commonly measured pro-inflammatory mRNAs, \textit{(Crp, Tnf, Il1b, and Ccl2)} (Figure 2). For Crp, HF-fed mice had significantly higher \textit{Crp} mRNA levels compared with the other treatments (Fig. 2A). The dietary groups showed no significant differences regarding the expression of \textit{Tnf} and \textit{Il1b} (Figs. 2B and 2C). For the case of \textit{Ccl2}, HF-fed mice had greater mRNA levels vs. LF-fed mice, and the WR-fed mice had mRNA levels reduced such that it was statistically indistinguishable from LF-fed mice (Fig. 2D).
4 Discussion

In our prior report (19), watermelon flesh intake, when consumed in the diet at a typical level (8% of total energy) improved parameters associated with glucose metabolism, and reduced levels of pro-inflammatory fatty acids in the liver. Consumption of high-fiber watermelon rind (2.25% w/w) also improved glucose metabolism, serum insulin, and food efficiency, while watermelon skin (2.25% w/w) and rind improved microbiome composition.

In the present study, we evaluated the impact of the inclusion of watermelon flesh and fiber-rich rind and skin by-products on hepatic gene expression. The goal of the present work was to identify mechanistic regulatory factors and pathways that are altered by our three test diet ingredients. There were a profound set of mRNAs differently regulated between LF- and HF-fed mice. Briefly, upon evaluation of the most up- and down-regulated canonical pathways, lipid metabolism, including both fatty acid, and sterol-related pathways were robustly upregulated. We suggest the prominent activation of these pathways is consistent with up-regulation of both β-oxidation and alterations of bile acid metabolism.

When a collection of mRNAs regulated by ligand-dependent transcription factors, such as PPARα and PXR was evaluated, we identified a very strong relationship between watermelon intake and PPARα regulation. This relationship alone would suggest that watermelon consumption is delivering a dietary compound, or a compound biotransformed by the microbiome, that is acting to agonize to PPARα-regulated gene transcription. Strongly supporting this hypothesis is the identification of $Cyp4a10$ and $Cyp4a14$ as the two most upregulated genes in the liver of WF-fed vs. HF-fed mice, as the Cyp4a family of genes is known to be strongly upregulated by PPARα. Other mRNAs found to be most strongly up-regulated with WF intake are $Acot3$, involved in conversion of acyl-coA molecules into free
fatty acids, *Abcd2*, which mediates peroxisome lipid import (26), and *Abcb1a*, an important xenobiotic and sterol plasma membrane transporter (27).

Further evidence of bioactive compounds from watermelon impacting liver function are indicated by the relatively large number of mRNAs up-regulated by the xenobiotic sensing receptors CAR and PXR. For example, four of the six most up-regulated mRNAs in WS-fed mice are known to be regulated by PXR and/or CAR. Up-regulation of xenobiotic metabolism implies that bile acid metabolism is being altered, as many of the phase I and II enzymes and plasma membrane transporters are shared for both xenobiotic compounds and bile acids (27). A second way that bile acid and sterol metabolism may be altered is by the fiber-rich WR and WS products – dietary fiber from these powders may be acting as a bile acid sequestrant. Although not a part of this study, it would be of interest in the future to measure fecal elimination of bile acids to determine if there is any significant impact.

Also of note was the determination that circadian regulation and rhythm are the two most up-regulated pathways associated with WR intake. Although not fully understood, present knowledge suggests circadian pathways are regulated, in part, by the Retinoic Acid-related Orphan Receptor (ROR), and there is likely some crosstalk between circadian rhythms and lipid metabolism. One notable example is *Cyp7b1*: this mRNA is known to be down-regulated by the ROR receptor and plays a significant role in sterol metabolism (28). The *Per3* gene plays an important role in the establishment of circadian phenotypes and rhythm disturbances, as well as being related to homeostatic sleep regulation, but the mechanism by which its function establishes these phenotypes and processes is not yet well understood. The circadian clock programs daily rhythms and coordinates multiple behavioral and physiological processes, including activity, sleep, feeding, and fuel homeostasis. The consumption of a high-calorie diet alters the function of the mammalian circadian clock (29). This demonstrates that along with the lipid-rich diet, watermelon products regulate circadian
rhythm. A possible cause of increased *Per3* gene expression is the decrease in non-esterified fatty acids (NEFA), which was previously reported by another study in our lab (19).

For the case of down-regulated canonical pathways, nearly every one of the most regulated pathways for WF/HF are energy-related processes. Protein folding and response to endoplasmic reticulum stress are also in the top 20 down-regulated pathways. For the case of WR/HF, most of the top 20 pathways relate to down-regulation of the unfolded protein response pathway (30,31), with three other pathways related to inflammation and the immune response. This finding strengthens the conclusions of our prior study Becraft et al (19) reporting reduced levels of pro-inflammatory fatty acids in the liver, as determined by metabolomic analysis.

Canonical pathways related to endoplasmic reticulum stress and the unfolded protein response (UPR) were robustly down-regulated in the WR group. Endoplasmic reticulum stress can be sensed through the composition of lipids in the endoplasmic reticulum, which is modified through lipid metabolism and activates SREBP2 (Sterol Regulatory Element Binding Protein-2) (32). Hypoxia is another source of endoplasmic reticulum stress that can induce UPR, which can occur as a consequence of excess lipid accumulation in cells, and is implicated in the pathology of obesity and NAFLD (33,34,35). The unfolded protein response regulates EIF2α (Eukaryotic translation Initiation Factor 2A), a major regulator of translation in eukaryotes that plays a critical role in the circadian rhythm regulation of mRNA translation (36,37), and thus a post-transcriptional regulator of cellular metabolism. The substantial downregulation of endoplasmic reticulum stress and UPR uniquely found in the WR-fed group further support the notion that constituents of watermelon rind are impacting the regulation of circadian rhythm, which may assist in the physiological stress response to increased adiposity.
For WS/HF, at least eight other pathways were related to down-regulation of vasoconstriction. This supports a large body of prior work demonstrating the hypotensive effect of watermelon consumption(38). Interestingly, there are both unique and overlapping pathways regulated with intake of WF, WR, and WS.

We evaluated a set of ligand-regulated regulatory factors (AhR, CAR, FXR, LXR, Nrf2, PPARα, PPARγ, and PXR) known to play important roles in lipid, glucose, and xenobiotic metabolism. The mRNAs listed in Tables 7-9 were included if they were significant or trended to significance in the WF, WR, or WS groups, either up or down vs. the HF-fed group, and are considered to be regulated by one of the ligand-regulated factors listed above. The most common factor in all three lists of genes was PPARα, suggesting these three diets are all improving lipid metabolism via PPARα-induced changes in β-oxidation and other lipid catabolic processes. Consumption of WF and WS appeared to impact the xenobiotic-related factors (Ahr, CAR, and PXR) more profoundly than WR.

Other factors that may be impacted by the watermelon products include LXR and FXR. For example, the sterol transporters *Abcg5* and *Abcg8* were significantly upregulated in mice eating all three watermelon diets. Liver X Receptors are master regulators of hepatobiliary reserve cholesterol transport (RCT), which is one route for cholesterol elimination from the body. LXR activation during feeding induces fatty acid synthesis and cholesterol transport, and its targets include ATP binding cassette (ABC) proteins, and the pro-lipogenic transcription factor *Srebp-1c*, as well as proteins involved in lipid remodeling such as cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), and lipoprotein lipase (LPL). Our results showed a response consistent with LXR activation by all the watermelon products (Table 2, 3, and 4) compared to HF diet (16,39).

Post-prandial hepatic activation of PXR and CAR promotes the clearance of toxic dietary metabolites, drugs, and xenobiotics through phase I, II, and III xenobiotic metabolism
CAR and PXR are activated by many different phytochemicals. In this study, these receptors were also activated by WF- and WS-diets, and to a lesser degree, WR-diet. The mRNAs regulated by AhR and Nrf2 generally follow this same pattern. It is also possible that activity of RXR (Retinoid X Receptor) may be regulated by one or more components of watermelon. If true, this could explain regulation typically ascribed to the RXR heterodimer partners, the PPARs, LXR, FXR, PXR, and CAR, but does not explain regulation observed for AhR and Nrf2.

PPARα has a critical role in the regulation of fatty acid uptake, beta-oxidation, ketogenesis, bile acid synthesis, and triglyceride turnover. Hepatic PPARα expression is low in NAFLD and increases in response to diet and exercise therapy. Implications of PPARα activation include suppression of inflammation in the obese state through complex regulation of NF-κB (40) and activator protein 1 (Ap1) transcription factors, and coordinate metabolism via transcription of the adipokine, adiponectin (by PPARγ), and along with FXR, on the hepatokine, Fgf21 (15).

Our results showed gene expression changes consistent with PPARα regulation in all three watermelon diets. Many ligands have been presented as possible agonists of PPARα. The natural compounds proposed as activators for PPARα, long-chain fatty acids, including polyunsaturated fatty acids such as linoleic acid, linolenic acid, eicosapentaenoic acid (EPA), and arachidonic acid, as well as derivatives of these fatty acids (41). The fatty acids responsible for activating these transcription factors may be directly available in watermelon or by other non-lipid phytochemicals.

WF, WR, and WS contained 4.0, 46.2, and 64.5 g/100 g of dietary fiber, respectively, (19). The physicochemical characteristics of fibers include fermentability, solubility, and viscosity. These properties influence not only fermentation, but also the therapeutic effects of consumption. Bacterial fermentation of polysaccharides results in the production of acidic
fermentation end products, primarily lactic acid and short chain fatty acids (SCFAs) such as butyrate, that reduce the colonic pH, which in turn impacts the composition of the microbial communities present in the gastrointestinal tract. Between 90 to 99% of SCFAs are absorbed in the gut or used by the microbiota (42), and thus these compounds may act as activators for PPARα.

Inflammation plays a key role in the development of atherosclerosis. One indicator of systemic inflammation is Crp levels (43). In our study, expression of Crp in WF-, WR-, and WS-fed mice was the same as measured in healthy LF-fed mice. Hong et al. (11) demonstrated decreased serum Crp concentration in male Sprague-Dawley rats after nine weeks of watermelon consumption. In their study, lower serum Crp levels were associated with upregulation of endothelial nitric oxide synthase (eNOS), which has a protective effect against atherosclerosis and inflammation. In combination, results from the present study, and data from Hong et al. (11) suggest watermelon intake is providing an anti-inflammatory effect.

The fact that WF, with a lower fiber content, impacts hepatic gene expression patterns significantly suggests another component besides fiber is bioactive. One likely candidate is lycopene, as WF has a relatively high lycopene content (~60 mg/100 g) (44). There is some evidence that lycopene impacts lipid metabolism and PPARα (45,46). Lycopene may also act via AhR signaling to reduce NASH progression in mice fed with high-fed diet (46).

Another component somewhat unique to watermelon is citrulline. It would be predicted that citrulline is mainly impacting physiology through the nitric oxide pathway and vasodilation (9). It is unclear if citrulline intake is impacting gene expression to any significant degree.
5 Conclusion

All three watermelon supplemented groups exhibited changes in gene expression patterns compared to HF-fed mice. Each of the three watermelon treatments had uniquely expressed genes compared with the other watermelon treatments, having 322, 99, and 62 differentially expressed genes for the WF, WR, and WS groups, respectively. These findings indicate that while all three watermelon products had significant impacts on the hepatic transcriptome, they each acted through both different and overlapping mechanisms, as a result of the unique phytochemical composition within each product. The actions exerted by the various diets may be through lycopene activating PPARα in WF, dietary fibers modulating the microbiota in WR (and thus acting less through the mechanism of hepatic nuclear receptors), and other phytochemicals acting on CAR in WS. It can be clearly seen, overall (Figure 3), how each watermelon component acts in these common and more unique pathways.

Our study utilized powdered watermelon products that were prepared by drying with heat. This method could be applied to prepare watermelon products to be used as functional foods or supplements in the future, and the results of this study reflect the consumption of such products. Utilization of watermelon byproducts as functional fiber supplements represents a sustainable and cost-effective value-added ingredient. Watermelon flesh, however, is generally consumed fresh and unprocessed. We did not assess the phytochemicals in the watermelon flesh powder, however it is likely that some loss and oxidation of various vitamins and phytochemicals may have occurred with processing. Vitamin C, lycopene, and polyunsaturated fatty acids are susceptible to oxidation, and their composition in the powder may not accurately reflect their representation in fresh watermelon. Because our processed samples likely had slightly decreased nutritional value, the fresh fruit may offer additional or more pronounced benefits than what was observed in this study.
7 Authors’ contributions to manuscript

N.F.S. designed research; M.B.E., G.P., A.R.B., M.S., W.Y, and N.F.S. conducted research; N.F.S., M.B.E., and G.P. analyzed data; and N.F.S., M.B.E., and G.P. wrote the paper. N.F.S. had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1. Hepatic Canonical Pathways most significantly up-regulated and down-regulated in male C57BL/6J mice fed HF (high fat) plus watermelon flesh (WF) vs. HF for 10 weeks

| Diet        | Canonical Pathways                                      | Number of genes | p-value       |
|-------------|---------------------------------------------------------|-----------------|---------------|
| WF/HF ↑     | fatty acid metabolic process                            | 51              | 2.90E-13      |
|             | extracellular matrix organization                       | 37              | 8.38E-13      |
|             | extracellular structure organization                    | 37              | 9.77E-13      |
|             | cell-substrate adhesion                                 | 45              | 2.18E-12      |
|             | regulation of cell-substrate adhesion                   | 29              | 7.43E-09      |
|             | positive regulation of protein kinase activity          | 47              | 1.39E-08      |
|             | positive regulation of kinase activity                  | 49              | 2.56E-08      |
|             | cell-matrix adhesion                                    | 28              | 2.61E-08      |
|             | microtubule cytoskeleton organization                   | 50              | 4.70E-08      |
|             | circadian rhythm                                        | 25              | 1.56E-06      |
|             | response to wounding                                    | 47              | 4.77E-08      |
|             | positive regulation of cell migration                   | 48              | 8.79E-08      |
|             | positive regulation of cell motility                    | 49              | 1.12E-07      |
|             | collagen fibril organization                            | 12              | 2.49E-07      |
|             | positive regulation of cellular component movement      | 49              | 2.71E-07      |
|             | lipid homeostasis                                       | 20              | 2.97E-07      |
|             | circadian regulation of gene expression                 | 14              | 3.98E-07      |
|             | posttranscriptional regulation of gene expression        | 41              | 5.39E-07      |
|             | lipid localization                                      | 37              | 6.45E-07      |
|             | positive regulation of protein serine/threonine kinase activity | 33              | 1.41E-06      |
| WF/HF ↓     | purine nucleoside triphosphate metabolic process        | 56              | 1.66E-19      |
|             | purine ribonucleoside triphosphate metabolic process    | 55              | 1.70E-19      |
|             | ribonucleoside triphosphate metabolic process           | 55              | 3.83E-19      |
|             | ATP metabolic process                                   | 51              | 7.35E-19      |
|             | nucleoside triphosphate metabolic process              | 56              | 3.59E-18      |
|             | purine ribonucleoside monophosphate metabolic process   | 53              | 5.43E-18      |
|             | purine nucleoside monophosphate metabolic process       | 53              | 6.59E-18      |
|             | ribonucleoside monophosphate metabolic process          | 53              | 9.65E-18      |
|             | nucleoside monophosphate metabolic process             | 53              | 4.26E-17      |
|             | protein folding                                         | 40              | 9.35E-17      |
|             | cytoplasmic translation                                 | 23              | 1.89E-14      |
|             | oxidative phosphorylation                               | 27              | 2.27E-14      |
|             | mitochondrial ATP synthesis coupled electron transport   | 22              | 8.81E-14      |
|             | purine nucleoside triphosphate biosynthetic process     | 23              | 1.03E-13      |
|             | ribosome biogenesis                                     | 48              | 2.42E-13      |
|             | ATP synthesis coupled electron transport                | 22              | 4.69E-13      |
|             | purine ribonucleoside triphosphate biosynthetic process | 22              | 6.95E-13      |
|             | response to endoplasmic reticulum stress                | 42              | 1.51E-12      |
|             | ribose phosphate metabolic process                      | 69              | 1.60E-12      |
|             | electron transport chain                                | 25              | 2.02E-12      |
Table 2. Hepatic Canonical Pathways most significantly up-regulated and down-regulated in male C57BL/6J mice fed HF (high fat) plus watermelon rind (WR) vs. HF for 10 weeks

| Diet        | Canonical Pathways                                | Number of genes | p-value     |
|-------------|---------------------------------------------------|-----------------|-------------|
| WR/HF ↑     | circadian regulation of gene expression           | 13              | 8.68E-10    |
|             | circadian rhythm                                  | 20              | 1.40E-08    |
|             | steroid metabolic process                         | 23              | 9.18E-08    |
|             | rhythmic process                                  | 24              | 1.87E-07    |
|             | regulation of circadian rhythm                    | 13              | 1.26E-06    |
|             | small molecule biosynthetic process               | 29              | 1.51E-06    |
|             | cellular carbohydrate metabolic process           | 20              | 1.60E-06    |
|             | RNA splicing                                      | 24              | 1.82E-06    |
|             | organic hydroxy compound metabolic process        | 27              | 2.26E-06    |
|             | monosaccharide metabolic process                  | 19              | 3.62E-06    |
|             | mRNA processing                                   | 26              | 4.48E-06    |
|             | sterol metabolic process                          | 13              | 5.51E-06    |
|             | glucose metabolic process                         | 16              | 6.13E-06    |
|             | cell-substrate adhesion                           | 21              | 7.29E-06    |
|             | cellular response to organic cyclic compound      | 27              | 8.01E-06    |
|             | fatty acid metabolic process                      | 23              | 8.41E-06    |
|             | sterol homeostasis                                | 10              | 8.50E-06    |
|             | cholesterol homeostasis                           | 10              | 8.50E-06    |
|             | cholesterol metabolic process                     | 12              | 1.20E-05    |
|             | liver morphogenesis                               | 6               | 1.39E-05    |
|             | response to unfolded protein                      | 14              | 1.32E-09    |
|             | response to topologically incorrect protein       | 15              | 1.90E-09    |
|             | response to endoplasmic reticulum stress          | 19              | 1.04E-08    |
|             | endoplasmic reticulum unfolded protein response   | 10              | 1.36E-07    |
|             | cellular response to unfolded protein             | 10              | 3.30E-07    |
|             | cellular response to topologically incorrect protein| 11              | 3.54E-07    |
|             | protein folding                                   | 14              | 4.97E-07    |
|             | acute-phase response                              | 8               | 5.82E-07    |
|             | acute inflammatory response                       | 11              | 3.76E-06    |
|             | regulation of neuron death                        | 19              | 1.00E-05    |
| WR/HF ↓     | proteasomal protein catabolic process             | 20              | 2.13E-05    |
|             | regulation of neuron apoptotic process            | 15              | 3.50E-05    |
|             | neuron death                                      | 19              | 3.89E-05    |
|             | response to oxidative stress                      | 19              | 3.89E-05    |
|             | activation of immune response                     | 17              | 4.37E-05    |
|             | response to inorganic substance                   | 19              | 5.35E-05    |
|             | intrinsic apoptotic signaling pathway             | 16              | 5.39E-05    |
|             | negative regulation of blood vessel diameter      | 9               | 7.77E-05    |
|             | 3'-UTR-mediated mRNA stabilization                | 4               | 8.11E-05    |
|             | neuron apoptotic process                          | 15              | 9.56E-05    |
Table 3. Hepatic Canonical Pathways most significantly up-regulated and down-regulated in male C57BL/6J mice fed HF (high fat) plus watermelon skin (WS) vs. HF for 10 weeks

| Diet       | Canonical Pathways                        | Number of genes | p-value         |
|------------|-------------------------------------------|-----------------|----------------|
| WS/HF ↑    | fatty acid metabolic process              | 23              | 3.04E-15       |
|            | lipid localization                        | 19              | 9.97E-12       |
|            | lipid transport                           | 18              | 1.19E-11       |
|            | regulation of lipid localization          | 11              | 7.54E-09       |
|            | fatty acid biosynthetic process           | 10              | 3.26E-08       |
|            | monocarboxylic acid biosynthetic process  | 11              | 8.13E-08       |
|            | positive regulation of lipid metabolic process | 10          | 1.47E-07       |
|            | organic anion transport                    | 15              | 1.55E-07       |
|            | regulation of plasma lipoprotein particle levels | 7              | 3.18E-07       |
|            | regulation of lipid metabolic process     | 13              | 6.13E-07       |
|            | collagen fibril organization              | 6               | 7.41E-07       |
|            | cholesterol transport                      | 7               | 1.04E-06       |
|            | sterol transport                          | 7               | 1.15E-06       |
|            | thioester metabolic process               | 7               | 1.39E-06       |
|            | acyl-CoA metabolic process                | 7               | 1.39E-06       |
|            | intestinal cholesterol absorption          | 4               | 1.53E-06       |
|            | regulation of lipid transport             | 8               | 1.63E-06       |
|            | response to acid chemical                 | 11              | 1.64E-06       |
|            | intestinal lipid absorption               | 4               | 2.19E-06       |
|            | small molecule biosynthetic process       | 15              | 2.90E-06       |
|            | response to unfolded protein              | 8               | 5.80E-08       |
|            | negative regulation of blood vessel diameter | 8             | 9.34E-08       |
|            | response to topologically incorrect protein | 8            | 2.54E-07       |
|            | protein activation cascade                | 6               | 7.01E-07       |
|            | Vasoconstriction                          | 7               | 1.01E-06       |
|            | protein folding                           | 8               | 1.99E-06       |
|            | regulation of blood vessel diameter       | 8               | 2.53E-06       |
|            | regulation of tube diameter               | 8               | 2.53E-06       |
|            | regulation of vasoconstriction            | 6               | 3.06E-06       |
|            | regulation of blood vessel size           | 8               | 4.19E-06       |
| WS/HF ↓    | positive regulation of vasoconstriction   | 5               | 4.23E-06       |
|            | regulation of tube size                   | 8               | 4.38E-06       |
|            | vascular process in circulatory system     | 8               | 1.12E-05       |
|            | response to calcium ion                   | 6               | 2.01E-05       |
|            | endoplasmic reticulum unfolded protein response | 5             | 2.52E-05       |
|            | positive regulation of heterotypic cell-cell adhesion | 3             | 2.59E-05       |
|            | response to endoplasmic reticulum stress   | 8               | 2.70E-05       |
|            | cellular response to unfolded protein      | 5               | 3.92E-05       |
|            | regulation of epithelial cell apoptotic process | 5             | 6.25E-05       |
|            | regulation of hormone levels              | 11              | 6.29E-05       |
Table 4. Top 20 up and downregulated hepatic mRNAs from male C57BL/6J mice fed HF (high fat) plus watermelon flesh (WF) vs. HF for 10 weeks

| No | Genes        | Log2Fold | Biological Process            | Genes        | Log2Fold | Biological Process            |
|----|--------------|----------|-------------------------------|--------------|----------|-------------------------------|
| 1  | Acot3        | 2.96     | fatty acid metabolism         | Ugt2b38      | -2.65   | transferase activity          |
| 2  | Abcd2        | 2.37     | fatty acid metabolism         | Mup17        | -2.03   | pheromone activity            |
| 3  | Abcb1a       | 2.08     | fatty acid metabolism         | Capn8        | -1.99   | protease activity             |
| 4  | Mki67        | 2.06     | mitosis                       | Lars2        | -1.83   | translation                   |
| 5  | F830016B08Rik| 1.97     | cytokine signaling            | Mup11        | -1.83   | pheromone activity            |
| 6  | Cyp4a10      | 1.86     | fatty acid metabolism         | Nlrp12       | -1.81   | inflammatory response         |
| 7  | Col3a1       | 1.75     | extracellular matrix          | Dhrs9        | -1.79   | sterol metabolism             |
| 8  | Col1a2       | 1.74     | extracellular matrix          | Cyp7b1       | -1.72   | sterol metabolism             |
| 9  | Col1a1       | 1.73     | extracellular matrix          | Selenbp2     | -1.67   | selenium transport            |
| 10 | Cyp2a22      | 1.72     | fatty acid metabolism         | Ces4a        | -1.66   | xenobiotic metabolism         |
| 11 | Cyp4a14      | 1.72     | fatty acid metabolism         | Mt1          | -1.65   | antioxidant activity          |
| 12 | Vldlr        | 1.69     | lipid metabolism              | Ces2b        | -1.63   | xenobiotic metabolism         |
| 13 | Ptgds        | 1.69     | Eicosanoid metabolism         | Slec22a28    | -1.61   | organic anion transport       |
| 14 | Slec22a29    | 1.61     | organic anion transport       | Mup1         | -1.59   | glucose and lipid metabolism  |
| 15 | Osbpl5       | 1.58     | cholesterol homeostasis       | Mup20        | -1.59   | pheromone activity            |
| 16 | Cal6a1       | 1.55     | extracellular matrix          | Elovl3       | -1.57   | fatty acid metabolism         |
| 17 | Tnc          | 1.54     | extracellular matrix          | Prtn3        | -1.54   | extracellular matrix          |
| 18 | Mmd2         | 1.53     | RAS signaling                 | Steap4       | -1.51   | adipocyte function            |
| 19 | Neat1        | 1.52     | transcriptional regulation    | Socs3        | -1.49   | cytokine signaling            |
| 20 | Gpc6         | 1.51     | cellular growth               | Avp4a        | -1.47   | vasopressin signaling         |
Table 5. Top 20 up and downregulated hepatic mRNAs from male C57BL/6J mice fed HF (high fat) plus watermelon (WR) vs. HF for 10 weeks

| No | Genes         | Log2Fold | Biological Process          | Genes         | Log2Fold | Biological Process          |
|----|---------------|----------|-----------------------------|---------------|----------|-----------------------------|
| 1  | Ptgsd         | 2.31     | eicosanoid metabolism       | Slc41a2       | -1.48    | magnesium transport         |
| 2  | Rgr           | 2.02     | retinoid metabolism         | Cxcl1         | -1.44    | chemokine signaling         |
| 3  | F830016B08Rik | 1.98     | cytokine signaling          | Cdkn1a        | -1.30    | cell cycle                  |
| 4  | Fbf1          | 1.69     | mitosis                     | Mt1           | -1.29    | antioxidant activity        |
| 5  | Abcd2         | 1.56     | fatty acid metabolism       | Inhbb         | -1.27    | TGF-β signaling             |
| 6  | Rlbp1         | 1.54     | retinoid metabolism         | Capn8         | -1.20    | membrane trafficking        |
| 7  | Igfhp5        | 1.54     | IGF signaling               | Gstp2         | -1.18    | cell cycle regulation       |
| 8  | Per3          | 1.52     | circadian rhythm            | Sdf2l1        | -1.17    | endoplasmic reticulum function |
| 9  | Tyrp1         | 1.46     | melanin metabolism          | Syt12         | -1.15    | neuronal signalling         |
| 10 | Pcsk4         | 1.41     | steroid processing          | Hspa1b        | -1.10    | protein folding             |
| 11 | H19           | 1.38     | gene expression             | Cyb561        | -1.08    | electron transport          |
| 12 | Igfbp8        | 1.34     | cell-cell interactions      | Fgg           | -1.07    | extracellular matrix        |
| 13 | Erbb4         | 1.26     | cell growth and differentiation | Steap4        | -1.07    | electron transport          |
| 14 | Ciaat         | 1.24     | circadian rhythm            | Gstp1         | -1.05    | xenobiotic metabolism       |
| 15 | Tnxb          | 1.23     | extracellular matrix        | Sdr9c7        | -1.04    | retinoid metabolism         |
| 16 | mt-Nd6        | 1.22     | NADH dehydrogenase          | S100a8        | -1.04    | inflammation and immune response |
| 17 | Tk1           | 1.22     | DNA replication             | Chrm3         | -1.03    | muscarinic acetylcholine signaling |
| 18 | Abcb1a        | 1.20     | xenobiotic efflux           | Cadm4         | -1.03    | cell-cell adhesion          |
| 19 | Chil1         | 1.18     | immune response             | H2afx         | -1.02    | nucleosome structure        |
| 20 | Col8a1        | 1.18     | extracellular matrix        | Saa1          | -1.01    | cholesterol homeostasis     |
Table 6. Top 20 up and downregulated hepatic mRNAs from male C57BL/6J mice fed HF (high fat) plus watermelon skin (WS) vs. HF for 10 weeks

| No | Genes | Log2Fold | Biological Process          | Genes | Log2Fold | Biological Process          |
|----|-------|----------|-----------------------------|-------|----------|-----------------------------|
| 1  | Acot3 | 1.84     | fatty acid metabolism       | Lars2 | -2.02    | mitochondrial translation   |
| 2  | H2-Q1 | 1.37     | immune response             | Dhrs9 | -1.42    | steroid metabolism         |
| 3  | Osbp3 | 1.36     | cellular structure          | Scara5| -1.37    | iron homeostasis            |
| 4  | Gstm2 | 1.35     | xenobiotic metabolism       | Egfr  | -1.15    | cellular growth             |
| 5  | Cidec | 1.29     | adipocyte metabolism        | Avpr1a| -1.09    | vasopressin signaling       |
| 6  | Colla1| 1.28     | extracellular matrix        | Slc41a2| -1.00    | magnesium transport         |
| 7  | Colla2| 1.24     | extracellular matrix        | Slc30a10| -0.99   | manganese homeostasis       |
| 8  | Tceal8| 1.19     | transcription               | Fgg   | -0.93    | extracellular matrix        |
| 9  | Lipg  | 1.19     | phospholipid metabolism     | Hspa5 | -0.92    | protein folding             |
| 10 | Pppl1r3g| 1.17    | glycoprotein metabolism     | Enho  | -0.90    | energy homeostasis          |
| 11 | Dpt   | 1.14     | extracellular matrix        | Socs3 | -0.90    | cytokine signaling          |
| 12 | Abcb1a| 1.14     | xenobiotic efflux            | Atp11a| -0.86    | phospholipid metabolism     |
| 13 | Acot2 | 1.13     | fatty acid metabolism       | Irf6  | -0.86    | transcriptional regulation  |
| 14 | Pcsk4 | 1.12     | steroid processing           | Cd163 | -0.86    | heme metabolism             |
| 15 | Krt14 | 1.12     | keratin processing          | Cyp2c70| -0.86    | bile acid metabolism        |
| 16 | Ciart | 1.08     | circadian rhythm            | Fgb   | -0.85    | extracellular matrix        |
| 17 | Gal3t1| 1.07     | sphingolipid metabolism     | Cyp2c54| -0.85    | eicosanoid metabolism       |
| 18 | Gpc6  | 1.07     | cellular growth             | Junb  | -0.84    | transcriptional regulation  |
| 19 | Fads3 | 1.06     | fatty acid metabolism       | Sdr9c7| -0.84    | retinoid metabolism         |
| 20 | Susd2 | 1.06     | oncogene                    | Slc3a1| -0.83    | amino acid transport        |
Table 7. Most significantly regulated hepatic mRNAs and associated ligand activated transcription factors from male C57BL/6J mice fed high fat (HF) plus watermelon flesh (WF) vs. HF diet for 10 weeks

| No | log2 FoldChange | pvalue     | GeneName | Regulatory factors |
|----|-----------------|------------|----------|--------------------|
|    |                 |            |          | AhR   | CAR  | FXR | LXR | Nrf2 | PPARα | PPARγ | PXR |
| 1  | 2.96            | 1.47E-54   | Acot3    | X     |      |     |     |      |       |       |     |
| 2  | 1.86            | 6.89E-52   | Cyp4a10  | X     |      |     |     |      |       |       |     |
| 3  | 1.34            | 2.88E-13   | Acot2    | X     |      |     |     |      |       |       |     |
| 4  | 1.72            | 1.33E-8    | Cyp4a14  | X     |      |     |     |      |       |       |     |
| 5  | 1.08            | 6.83E-10   | Acot4    | X     |      |     |     |      |       |       |     |
| 6  | 0.28            | 5.41E-02   | Acot7    | X     |      |     |     |      |       |       |     |
| 7  | 1.06            | 1.89E-16   | Abcg8    | X     |      |     |     |      |       |       |     |
| 8  | 1.06            | 4.39E-04   | Slc10a4  | X     |      |     |     |      |       |       |     |
| 9  | 1.04            | 7.65E-05   | Ugt1a6a  | X     |      |     |     |      |       |       |     |
| 10 | 1.01            | 5.55E-13   | Cyp7a1   | X     |      |     |     |      |       |       |     |
| 11 | 0.93            | 4.77E-10   | Abcg5    | X     |      |     |     |      |       |       |     |
| 12 | 0.92            | 5.92E-04   | Gstm2    | X     | X    | X   |     |      |       |       |     |
| 13 | 0.87            | 3.70E-06   | Abcg1    | X     |      |     |     |      |       |       |     |
| 14 | 0.86            | 2.75E-05   | Cidea    | X     |      |     |     |      |       |       |     |
| 15 | 0.86            | 3.25E-03   | Gstm3    | X     | X    | X   | X   | X     |       |       |     |
| 16 | 0.86            | 2.95E-04   | Ugt1a9   | X     | X    | X   |     | X     |       |       |     |
| 17 | 0.84            | 1.75E-05   | Mgst3    | X     | X    |     |     |       |       |       |     |
| 18 | 0.80            | 6.49E-10   | Aldh3a2  | X     |      |     |     |      |       |       |     |
| 19 | 0.77            | 3.62E-09   | Abcc3    | X     | X    | X   | X   | X     |       |       |     |
| 20 | 0.74            | 1.20E-10   | Paps2    | X     |      |     |     |      |       |       |     |
| 21 | 0.74            | 7.06E-06   | Acot11   | X     |      |     |     |      |       |       |     |
| 22 | 0.74            | 1.23E-02   | Fasn     |      |      |     |     |      |       |       |     |
| 23 | 0.72            | 7.21E-03   | Gsta2    | X     |      |     |     |      |       |       |     |
| 24 | 0.71            | 4.05E-06   | Sreb1l1  | X     |      |     |     |      |       |       |     |
| 25 | 0.68            | 2.24E-02   | Cyp2b11  | X     | X    |     |     |      |       |       |     |
| 26 | 0.67            | 1.37E-02   | Abcc4    | X     | X    | X   |     |      |       |       |     |
| 27 | 0.64            | 3.40E-02   | Gsta1    | X     | X    | X   |     |      |       |       |     |
| 28 | 0.63            | 7.08E-07   | Abcc2    | X     | X    | X   |     |      |       |       |     |
| 29 | 0.62            | 3.17E-03   | Scd2     | X     |      |     |     |      |       |       |     |
| 30 | 0.60            | 1.12E-02   | Slc27a1  | X     |      |     |     |      |       |       |     |
| 31 | 0.57            | 2.63E-02   | Gstm1    | X     |     | X   |     |      |       |       |     |
| 32 | 0.57            | 3.59E-03   | Gsta2    | X     |      |     |     |      |       |       |     |
| 33 | 0.49            | 2.88E-03   | Acaca    | X     |      |     |     |      |       |       |     |
| 34 | 0.47            | 4.20E-02   | Acot9    | X     |      |     |     |      |       |       |     |
| 35 | 0.40            | 1.04E-03   | Mafg     | X     |      |     |     |      |       |       |     |
| 36 | 0.40            | 5.11E-03   | Scd1     | X     |      |     |     |      |       |       |     |
| 37 | 0.39            | 2.14E-03   | Abca1    | X     |      |     |     |      |       |       |     |
|   | p-value | log10(p-value) | Gene     |   |
|---|---------|----------------|----------|---|
| 38| 0.35    | 5.75E-04       | Slec2a2  | X|
| 39| 0.30    | 3.56E-03       | Acat12   | X|
| 40| 0.17    | 9.71E-02       | Aldh7a1  | X|
| 41| 0.21    | 7.32E-02       | Creb3l   | X|
| 42| 0.38    | 9.94E-04       | Ugt2a3   | X X|
| 43| 0.44    | 6.27E-02       | Tnfaip3  | X|
| 44| 0.45    | 1.35E-04       | Apoc3    | X|
| 45| 0.47    | 1.66E-06       | Apoa1    | X|
| 46| 0.62    | 2.56E-02       | Solec1a1 | X|
| 47| 0.81    | 6.29E-03       | Il1b     | X|
| 48| 1.11    | 3.39E-21       | Ugt2b1   | X X|
Table 8. Most significantly regulated hepatic mRNAs and associated ligand activated transcription factors from male C57BL/6J mice fed high fat (HF) plus watermelon rind (WR) compared with HF diet for 10 weeks

| No | log2 FoldChange | pvalue     | GeneName   | Regulatory factors |
|----|----------------|------------|------------|--------------------|
|    |                |            |            | AhR   | CAR | FXR | LXR | Nrf2 | PPARα | PPARγ | PXR |
| 1  | 1.09           | 7.28E-09   | Cyp7a1     |        |     |     |     |      |       |       | X   |
| 2  | 1.01           | 4.28E-09   | Abcg8      |        |     |     |     |      |       |       | X   |
| 3  | 0.95           | 7.16E-07   | Abcg5      |        |     |     |     | X     | X     |     |     |
| 4  | 0.96           | 2.48E-04   | Acot3      |        |     |     |     |       |       |       | X   |
| 5  | 0.90           | 2.24E-05   | Acot2      |        |     |     |     |       |       |       | X   |
| 6  | 0.90           | 2.41E-04   | Cyp46a1    |        |     |     |     |       |       |       | X   |
| 7  | 0.79           | 4.27E-03   | Cyp4a10    |        |     |     |     |       |       |       | X   |
| 8  | 0.75           | 9.45E-04   | Acot4      |        |     |     |     |       |       |       | X   |
| 9  | 0.75           | 2.44E-03   | Ugt1a6a    |        |     |     |     |       |       |       | X   |
| 10 | 0.74           | 5.42E-06   | Papss2     |        |     |     |     |       |       |       | X   |
| 11 | 0.66           | 1.47E-02   | Slco1a4    |        |     |     |     |       |       |       | X   |
| 12 | 0.57           | 3.43E-06   | Abcc2      |        |     |     |     |       |       |       | X   |
| 13 | 0.53           | 2.25E-03   | Gsdt2      |        |     |     |     |       |       |       | X   |
| 14 | 0.53           | 5.92E-03   | Gstm2      |        |     |     |     |       |       |       | X   |
| 15 | 0.53           | 1.51E-03   | Srebfl     |        |     |     |     |       |       |       |     |
| 16 | 0.53           | 2.55E-02   | Slc27a1    |        |     |     |     |       |       |       | X   |
| 17 | 0.51           | 2.56E-04   | Abcc3      |        |     |     |     |       |       |       | X   |
| 18 | 0.50           | 2.83E-03   | Acot11     |        |     |     |     |       |       |       | X   |
| 19 | 0.47           | 1.76E-03   | Aldh3a2    |        |     |     |     |       |       |       | X   |
| 20 | 0.43           | 6.65E-02   | Cyp4a14    |        |     |     |     |       |       |       | X   |
| 21 | 0.42           | 3.33E-03   | Abca1      |        |     |     |     |       |       |       | X   |
| 22 | 0.35           | 1.15E-02   | Slc2a2     |        |     |     |     |       |       |       | X   |
| 23 | 0.34           | 1.52E-02   | Acot12     |        |     |     |     |       |       |       | X   |
| 24 | 0.33           | 2.53E-02   | Mag1       |        |     |     |     |       |       |       | X   |
| 25 | 0.23           | 8.75E-02   | Gyk        |        |     |     |     |       |       |       | X   |
| 26 | -0.21          | 7.31E-02   | Creb3l3    |        |     |     |     |       |       |       | X   |
| 27 | -0.23          | 5.22E-02   | Apoc3      |        |     |     |     |       |       |       | X   |
| 28 | -0.72          | 2.50E-03   | Tnfaip3    |        |     |     |     |       |       |       | X   |
Table 9. Most significantly regulated hepatic mRNAs and associated ligand activated transcription factors from male C57BL/6J mice fed high fat (HF) plus watermelon skin (WS) diet compared with HF diet for 10 weeks

| No | log2FoldChange | pvalue   | GeneName | Regulatory factors |
|----|----------------|----------|----------|---------------------|
|    |                |          |          | AhR    | CAR   | FXR   | LXR   | Nrf2   | PPARα  | PPARγ  | PXR   |
| 1  | 1.84           | 6.51E-16 | Acot3    | X      |       |       |        |        |        |        |       |
| 2  | 1.35           | 1.76E-12 | Gstm2    | X      | X     |       |        |        |        |        |       |
| 3  | 1.29           | 1.02E-10 | Cidec    | X      |       |       |        |        |        |        |       |
| 4  | 1.13           | 2.47E-08 | Acot2    |         |       |       |        |        |        |        |       |
| 5  | 0.88           | 8.63E-05 | Ugt1a5   | X      |       |       |        |        |        |        |       |
| 6  | 0.79           | 2.49E-06 | Gstm1    | X      | X     | X     |        |        |        |        |       |
| 7  | 0.77           | 1.12E-03 | Cyp4a10  |         |       |       |        |        |        |        |       |
| 8  | 0.75           | 9.66E-04 | Gstm3    | X      | X     | X     | X      |        |        |        |       |
| 9  | 0.74           | 9.16E-07 | Abcg8    |         |       |       |        |        |        |        | X     |
| 10 | 0.73           | 1.90E-07 | Aldh3a2  |         |       |       |        |        |        |        | X     |
| 11 | 0.70           | 7.54E-04 | Aco4     |         |       |       |        |        |        |        | X     |
| 12 | 0.67           | 3.23E-03 | Cidea    |         |       |       |        |        |        |        | X     |
| 13 | 0.65           | 4.72E-03 | Cyp46a1  |         |       |       |        |        |        |        | X     |
| 14 | 0.65           | 4.08E-03 | Slec27a1 |         |       |       |        |        |        |        | X     |
| 15 | 0.62           | 2.06E-04 | Abcc3    | X      | X     | X     | X      |        |        |        |       |
| 16 | 0.57           | 1.00E-02 | Ugt1a9   | X      | X     | X     | X      |        |        |        |       |
| 17 | 0.54           | 7.32E-03 | Cyp7a1   |         |       |       |        |        |        |        | X     |
| 18 | 0.53           | 9.04E-05 | Apoc2    |         |       |       |        |        |        |        | X     |
| 19 | 0.53           | 1.49E-03 | Abcg5    | X      |       |       |        |        |        |        | X     |
| 20 | 0.50           | 3.03E-03 | Sreb1    |         |       |       |        |        |        |        | X     |
| 21 | 0.49           | 8.47E-03 | Cyp4a14  |         |       |       |        |        |        |        | X     |
| 22 | 0.48           | 4.21E-02 | Solec1a4 | X      |       |       |        |        |        |        | X     |
| 23 | 0.46           | 1.79E-02 | Sult1e1  | X      |       |       |        |        |        |        |       |
| 24 | 0.46           | 1.44E-03 | Papss2   | X      |       |       |        |        |        |        | X     |
| 25 | 0.44           | 6.77E-03 | Acot11   |         |       |       |        |        |        |        | X     |
| 26 | 0.41           | 1.51E-02 | Abcc3    | X      | X     | X     |        |        |        |        |       |
| 27 | 0.23           | 4.37E-02 | Slec2a2  |         |       |       |        |        |        |        | X     |
| 28 | 0.34           | 7.07E-02 | Mgst3    | X      | X     |       |        |        |        |        |       |
| 29 | 0.32           | 8.86E-02 | Gstt2    |         |       |       |        |        |        |        | X     |
| 30 | 0.30           | 7.32E-02 | Gyk      |         |       |       |        |        |        |        | X     |
| 31 | 0.27           | 4.81E-02 | Gstm4    | X      | X     | X     |        |        |        |        |       |
| 32 | 0.28           | 6.99E-02 | Acot7    |         |       |       |        |        |        |        | X     |
| 33 | -0.30          | 5.40E-02 | Ugt2a3   | X      |       |       |        |        |        |        | X     |
| 34 | -0.42          | 5.27E-02 | Slec1a1  | X      | X     |       |        |        |        |        | X     |
| 35 | -0.53          | 1.56E-02 | Tnfaip3  |         |       |       |        |        |        |        | X     |
| 36 | -0.56          | 2.20E-03 | Ugt2b1   | X      |       |       |        |        |        |        | X     |
| 37 | -0.82          | 6.10E-04 | Arntl    |         |       |       |        |        |        |        | X     |
Figure 1. Venn diagram with gene expression of liver from male C57BL/6J mice fed with each diet: (A) low fat (LF) (gray), high fat (HF) (blue), HF plus watermelon flesh (WF) (red), HF plus watermelon rind (WR) (pink), and HF plus watermelon skin (WS) diets (skin); (B) HF, WF, WR, and WS; (C) WF, WR, and WS; (D) LF, HF, and WF; (E) LF, HF, and WR; and (F) LF, HF, and WS.
Figure 2. *Crp*, *Tnf*, *Il1b*, and *Ccl2* cytokines expressed in fpkm (A, B, C, and D, respectively) of liver from male C57BL/6J mice fed with low fat diet (LF), high fat diet (HF), high fat diet plus watermelon flesh (WF), high fat diet plus watermelon rind (WR), and high fat diet plus watermelon skin (WS). Different letters indicate statistical significance between different diets by Tukey test (p<0.05).
**Figure 3.** Selected most significant down- and up-regulated hepatic canonical pathways in male C57BL/6J mice fed high fat diet plus watermelon flesh (WF), high fat diet plus watermelon rind (WR), and high fat diet plus watermelon skin (WS) compared to high-fat-(HF-) fed mice.