ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Genomics, Proteomics, and Metabolomics

Intake of Watermelon and Watermelon Byproducts in 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.

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

Methods: Hepatic RNA was isolated and RNA sequencing was performed following a 10-week feeding trial during which C57BL/6 J mice were provided either a low-fat diet (LF), high-fat diet (HF; controls), or HF plus either WS, WR, or WF. Bioinformatic approaches were used to determine changes in the canonical pathways and gene expression levels for lipid- and xenobiotic-regulating nuclear hormone receptors and other related transcription factors, including the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), farnesyl X receptor, peroxisome proliferator–activated receptor alpha (PPARα), peroxisome proliferator–activated receptor gamma, liver X receptor, pregnane X receptor, and nuclear factor erythroid 2–related factor 2.

Results: There were 9394 genes that had unchanged expression levels between all 5 diet groups, and 247, 58, and 34 genes were uniquely expressed in the WF, WR, and WS groups, respectively. The relative levels of mRNAs regulated by AhR, CAR, and PPARα were upregulated in mice in the WF group, as compared to the HF control mice; in comparison, mRNAs regulated mainly by CAR were upregulated in mice in the WR and WS groups, compared to those in the HF control group.

Conclusions: At modest levels of intake reflective of typical human consumption, mice in the WF, WS, and WR groups exhibited hepatic gene expression profiles that were altered when compared to mice in the HF control group. Several of these changes involve genes regulated by ligand-responsive transcription factors implicated in xenobiotic and lipid metabolisms, suggesting that the modulation of these transcription factors occurred in response to the consumption of WS, WR, and WF. Some of these changes are likely due to nuclear hormone receptor–mediated changes involved in lipid and xenobiotic metabolisms. *Curr Dev Nutr* 2020;4:nzaa122.

Keywords: watermelon, RNA sequencing, obesity, diabetes, metabolic syndrome, mice, nuclear hormone receptors

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Abbreviations used: AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; CRP, C-reactive protein; eNOS, endothelial nitric oxide synthase; FXR, farnesyl X receptor; HF, high-fat diet; LF, low-fat diet; LXR, liver X receptor; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; Nrf2, nuclear factor erythroid 2–related factor 2; PPARα, peroxisome proliferator–activated receptor alpha; PPARγ, peroxisome proliferator–activated receptor gamma; PXR, pregnane X receptor; ROR, retinoic acid–related orphan receptor; RXR, retinoid X receptor; UPR, unfolded protein response; WF, watermelon flesh; WR, watermelon rind; WS, watermelon skin.

Introduction

Metabolic syndrome is a condition that affects a quarter of the global population; it is defined as the co-occurrence of multiple metabolic risk factors, including insulin resistance, hyperinsulinemia, impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, and visceral obesity (1, 2), which substantially increase an individual's risk of developing nonalcoholic 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 the development and progression of NAFLD and NASH (5). Further

evidence suggests that beyond low-carbohydrate and low-fat approaches, the inclusion of vitamins C, D, and E, as well as antioxidant compounds, from fruit intake may have a protective effect (6, 7). Research has shown that the inclusion of fruits or their extracts can ameliorate some of the negative metabolic consequences of a Western-style diet by decreasing the deposition of fat in the liver and modulating the activity of several transcription factors that regulate the metabolism of lipids, such as peroxisome proliferator–activated receptor alpha (PPARα) and Sterol Response Element Binding Protein-1c (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 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 affect epigenetic changes to control transcription (15, 16). Ligand-activated nuclear hormone receptors may bind dietary compounds, contributing to the 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). The consumption of less than 400 g per day of fruit and vegetables, excluding potatoes and other root vegetables (17), results in a 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 [HF; 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 10-week-old male C57BL/6 J mice ($n = 12$ in the control group; $n = 8$ per supplemented group). The watermelon-supplemented diets improved fasting blood glucose levels and produced changes in hepatic metabolite accumulation, especially as related to fatty acid metabolism and inflammation. In the present study, we investigated the effect that the consumption of WF, WR, and 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.

Methods

Watermelon preparation

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

Mouse diet studies

We randomly divided 48 male C57BL/6 J mice (Jackson Laboratory) into 2 control groups ($n = 12$ each) and 3 experimental groups ($n = 8$ each) at 6 weeks of age. After a 4-week acclimatization period, the groups were provided with experimental diets for 10 weeks. The Western-style obesogenic mouse diet, also referred to as the HF, contains 45% kcal fat + 20% kcal sucrose + 1% (w/w) cholesterol. There were 2 control groups: the first was on a low-fat diet (LF; 10% kcal fat) and the second was on the HF diet. There were 3 treatment groups, which were each fed a HF diet supplemented with either dried WF at 8% total energy (kcal), WR at 2.25% (w/w), or WS at 2.25% (w/w; **Supplemental Table 1**). We balanced the macronutrients such that every HF-based diet contained the same total percentage of each macronutrient, but with different fiber and phytochemical contents depending on the foods added, as previously described by Becraft et al. (19). The WF was included in the diets at 8% of total energy, to model the human intake of 2 servings of watermelon per day (i.e., 160 calories in a 2000-calorie daily diet). The WR and WS 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, the mice were fasted for 5 hours, anesthetized with isoflurane, and euthanized via cardiac puncture followed by cervical dislocation. The liver tissue was collected, flash frozen on dry ice, and stored at -20°C in RNA-later reagent. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Oregon State University, and the experiments were approved by the Oregon State University Animal Care and Use Committee (Protocol #4455).

RNA sequencing

RNA extraction, library preparation, and sequencing.

The total liver RNA was extracted from the liver tissue (~50 mg) using Trizol reagent (Invitrogen Life Technologies), following the standard protocol. The quantity and quality of the isolated RNA were analyzed using a Nano Drop 2000 spectrophotometer (Thermo Scientific). The 3 samples from each group with the highest RNA Integrity Number (RIN), λ260/280, and λ260/230 scores were used for RNA sequencing. Sequencing libraries were generated using the NEBNext Ultra RNA Library Prep Kit for

TABLE 1 Hepatic canonical pathways most significantly upregulated and downregulated in high-fat diets with and without watermelon flesh

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

Data were in male C57BL/6 J mice fed HF plus WS vs. HF for 10 weeks. Abbreviations: HF, high-fat diet; WS, watermelon skin; ↑, upregulated; ↓, downregulated.

Illumina (NEB), following the manufacturer's standard protocols. The 6 constructed mRNA libraries were sequenced on an Illumina HiSeq 2000 (Illumina) at Novogene Technology Co., Ltd (Novogene Gene Technology).

Differential expression analysis.

The raw data (raw reads) were in FASTQ format and 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 the amplification of genomes, polynucleotides (poly-N), and low-quality reads (in which more than 50% of bases had a quality value ≤20) from the raw data. All downstream analyses were based on the cleaned data. Reads were obtained from Gene Expression Analysis to perform a differential expression analysis using the DESeq2 R package (2 conditions/groups), while the significant criterion was an adjusted *P* value of ≤0.05.

Global gene expression was compared between the HF control group and the WF, WR, and WS groups. To identify those genes likely to be direct targets of transcription factor–dependent regulation of hepatic receptors in male mice in response to the watermelon diets, we utilized genes known to be regulated by the aryl hydrocarbon receptor (AhR) (20), constitutive androstane receptor (CAR) (20), PPARα (20), peroxisome proliferator–activated receptor gamma (PPARγ) (21), nuclear factor erythroid 2–related factor 2 (Nrf2) (20), pregnane X receptor (PXR) (20, 22), liver X receptor (LXR) (23, 24), and farnesyl X receptor (FXR) (25), as described in the literature (**Supplemental Table 2**). Due to the multiple differences in the 2 control diets, we chose not to detail the differences between LF and HF mice in this report.

TABLE 2 Hepatic canonical pathways most significantly upregulated and downregulated in high-fat diets with and without watermelon rind

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
WR/HF ↓	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
	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′-untranslated region–mediated mRNA stabilization	4	8.11E-05
	neuron apoptotic process	15	9.56E-05

Data were in male C57BL/6 J mice fed HF plus WR vs. HF for 10 weeks. Abbreviations: HF, high-fat diet; WR, watermelon rind; ↑, upregulated; ↓, downregulated.

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

Results

Previously, we evaluated WF, WR, and WS diets in male C57BL/6 J mice fed a Western-style obesogenic diet. We reported that the average body weight was significant higher in HF-fed mice than LF-fed mice, and no weight difference was found between any watermelon group and the HF control group. The energy intake was highest in mice in the WS group, followed those in the HF control and WR groups, and was lowest in mice in the LF group. The energy efficiency was higher in mice in the WR and WS groups than in mice in the LF, HF control, and WF groups. Mice in the WF, WS, and WS groups had reduced fasting blood glucose concentrations, which did not significantly differ from those of the LF and HF control groups. The WR group showed a lower insulin concentration than the HF control group, while the WF and WS groups showed no significant difference from the HF control group. As for serum biomarker quantification, Macrophage Chemoattractant Protein-1 (MCP-1) was significantly lower in the WS group than in the HF control group, and no significant differences were observed for resistin concentrations. A metabolomic analysis demonstrated that a set of liver lipid species were changed with the consumption of watermelon products. Further, cecal bacteria populations from WS-fed mice shifted toward those of LF-fed mice. Thus, when compared to mice in the HF control group, obese mice in each of the WS, WF, and WR groups showed improved fasting blood glucose levels, circulating serum

TABLE 3 Hepatic canonical pathways most significantly upregulated and downregulated in high-fat diets with and without watermelon skin

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
WS/HF ↓	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
	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

Data were in male C57BL/6 J mice fed HF plus WS vs. HF for 10 weeks. Abbreviations: HF, high-fat diet; WS, watermelon skin; ↑, upregulated; ↓, downregulated.

insulin concentrations, and/or changes in hepatic metabolite accumulation (19).

In the present work, to characterize the effect of 10 weeks of a dietary intervention on global liver gene expression, the transcriptomes of all groups of 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% (**Supplemental Table 3**). A total of 20,349 different transcripts were analyzed (**Figure 1**). Across all 5 diet groups, there were 9394 transcripts that had unchanged expression levels (Figure 1A). There were 78, 34, 247, 58, and 34 genes uniquely expressed in the LF, HF control, WF, WR, and WS groups, respectively (Figure 1A and B). When the 3 watermelon groups were compared, there were 9811 genes that had unchanged expression levels in the 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 control group), both up and down, are shown in Tables 1–3. For all 3 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 2 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 5 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).

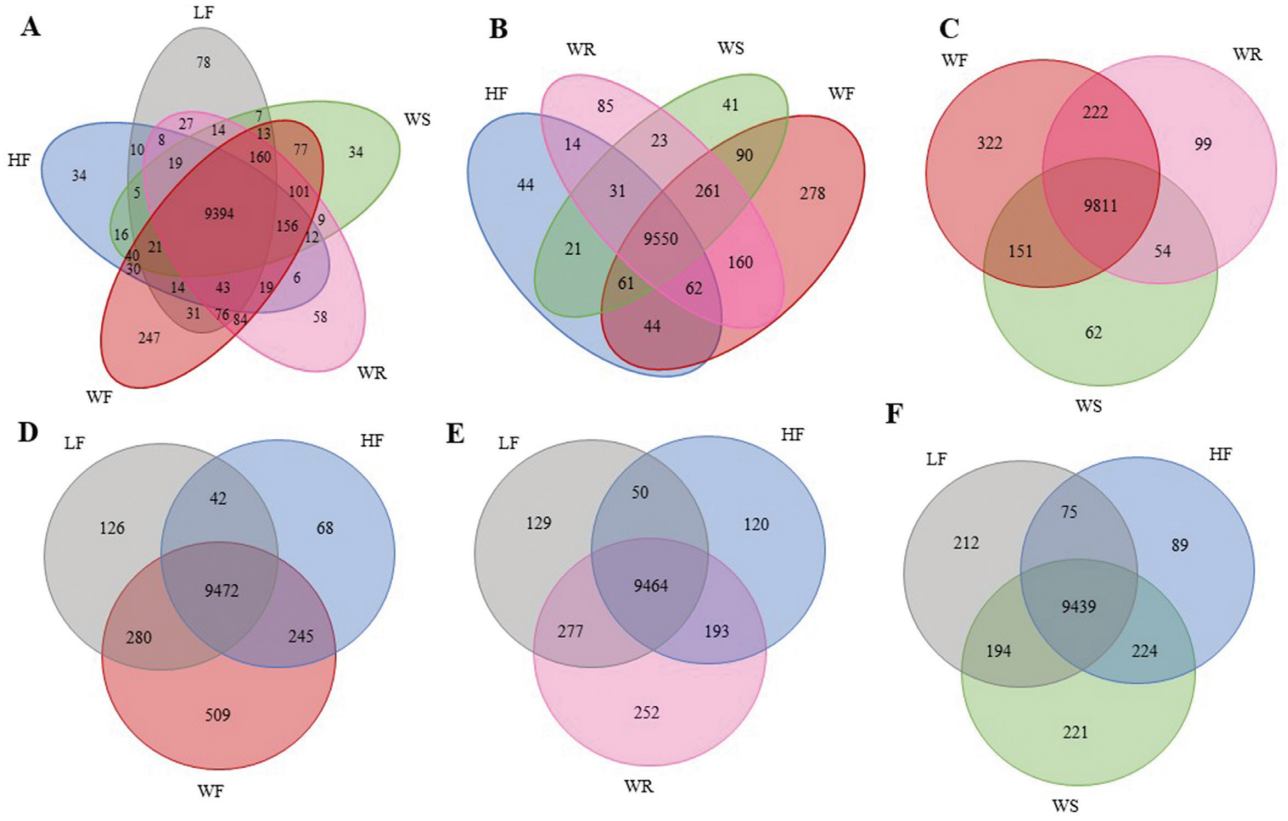

FIGURE 1 Venn diagram with gene expression of liver from male C57BL/6 J mice fed with each diet: (A) LF (gray), HF (blue), HF plus WF (red), HF plus WR (pink), and HF plus WS (green); (B) HF, WF, WR, and WS; (C) WF, WR, and WS; (D) LF, HF, and WF; (E) LF, HF, and WR; and (F) LF, HF, and WS. Abbreviations: HF, high-fat diet; LF, low-fat diet; WF, watermelon flesh; WR, watermelon rind; WS, watermelon skin.

For the case of downregulated canonical pathways, nearly all 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 downregulated pathways. For the case of WR/HF, most of the top 20 pathways relate to downregulation of the unfolded protein response pathway, with 3 other pathways related to inflammation and the immune response. For WS/HF, the unfolded protein response is again prominent,

TABLE 4 Top 20 up- and downregulated hepatic mRNAs from high-fat diets with and without watermelon flesh

	Upregulated Genes			Downregulated Genes		
No	Genes	Log2 Fold Change	Biological Process	Genes	Log2 Fold Change	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	*Slc22a28*	-1.61	organic anion transport
14	*Slc22a29*	1.61	organic anion transport	*Mup1*	-1.59	glucose and lipid metabolism
15	*Osbpl5*	1.58	cholesterol homeostasis	*Mup20*	-1.59	pheromone activity
16	*Col6a1*	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	renin-angiotensin system signaling	*Steap4*	-1.51	adipocyte function
19	*Neat1*	1.52	transcriptional regulation	*Socs3*	-1.49	cytokine signaling
20	*Gpc6*	1.51	cellular growth	*Avpr1a*	-1.47	vasopressin signaling

Data were in male C57BL/6 J mice fed HF plus WF vs. HF for 10 weeks. Abbreviations: HF, high-fat diet; WF, watermelon flesh.

TABLE 5 Top 20 up- and downregulated hepatic mRNAs from high-fat diets with and without watermelon rind

	Upregulated Genes			Downregulated Genes		
No	Genes	Log2 Fold Change	Biological Process	Genes	Log2 Fold Change	Biological Process
1	*Ptgds*	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	*Igfbp5*	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 signaling
10	*Pcsk4*	1.41	steroid processing	*Hspa1b*	-1.10	protein folding
11	*H19*	1.38	gene expression	*Cyb561*	-1.08	electron transport
12	*Itgb8*	1.34	cell-cell interactions	*Fgg*	-1.07	extracellular matrix
13	*Erbb4*	1.26	cell growth and differentiation	*Steap4*	-1.07	electron transport
14	*Ciart*	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	NAD(H) 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

Data are from male C57BL/6 J mice fed HF plus WR vs. HF for 10 weeks. Abbreviations: HF, high-fat diet; IGF, insulin-like growth factor; TGF, transforming growth factor; WR, watermelon rind.

with at least 8 other pathways related to downregulation of vasoconstriction.

Lists of the 20 most up- and downregulated genes for the WF, WR, and WS groups, compared to the HF control group, 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), *Col1a2*

TABLE 6 Top 20 up- and downregulated hepatic mRNAs from high-fat diets with and without watermelon skin

	Upregulated Genes			Downregulated Genes		
No	Genes	Log2 Fold Change	Biological Process	Genes	Log2 Fold Change	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	*Osbpl3*	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	*Col1a1*	1.28	extracellular matrix	*Slc41a2*	-1.00	magnesium transport
7	*Col1a2*	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	*Ppp1r3g*	1.17	glycogen 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	*Krt23*	1.12	keratin processing	*Cyp2c70*	-0.86	bile acid metabolism
16	*Ciart*	1.08	circadian rhythm	*Fgb*	-0.85	extracellular matrix
17	*Gal3st1*	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

Data are from male C57BL/6 J mice fed HF plus WS vs. HF for 10 weeks. Abbreviations: HF, high-fat diet; WS, watermelon skin.

TABLE 7 Most significantly regulated hepatic mRNAs and associated ligand activated transcription factors from high-fat diets with and without watermelon flesh

No	Log2 Fold Change	*P* Value	Gene Name	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	*Slco1a4*		X						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	*Cidec*							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					X		
19	0.77	3.62E-09	*Abcc3*		X			X	X		X
20	0.74	1.20E-10	*Papss2*		X				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	*Srebf1*			X					
25	0.68	2.24E-02	*Cyp2b10*	X	X						
26	0.67	1.37E-02	*Abcc4*	X	X			X	X		
27	0.64	3.40E-02	*Gsta1*		X			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	X		X
32	0.57	3.59E-03	*Gstt2*						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				
38	0.35	5.75E-04	*Slc2a2*							X	
39	0.30	3.56E-03	*Acot12*						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	*Slco1a1*			X					
47	−0.81	6.29E-03	IL1B				X				
48	−1.11	3.39E-21	*Ugt2b1*	X	X						

Data are from male C57BL/6 J mice fed HF plus WF vs. HF diet for 10 weeks. Abbreviations: AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; FXR, farnesyl X receptor; HF, high-fat diet; LXR, liver X receptor; Nrf2, nuclear factor erythroid 2–related factor 2; PPARα, peroxisome proliferator–activated receptor alpha; PPARγ, peroxisome proliferator–activated receptor gamma; PXR, pregnane X receptor; WF, watermelon flesh.

(1.74-fold), and *Col1a1* (1.73-fold). The WR/HF expression ratios resulted in the 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 *Fbf1* (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 include 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, such as *Col1a1* (1.28-fold) and *Col1a2* (1.24-fold); and xenobiotic metabolism and transport-related genes, including *Gstm2* (1.35-fold) and *Abcb1a* (1.14-fold).

TABLE 8 Most significantly regulated hepatic mRNAs and associated ligand activated transcription factors from high-fat diets with and without watermelon rind

No	Log2 Fold Change	*P* Value	Gene Name	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				X		
11	0.66	1.47E-02	*Slco1a4*		X						X
12	0.57	3.43E-06	*Abcc2*		X	X			X		
13	0.53	2.25E-03	*Gstt2*						X		
14	0.53	5.92E-03	*Gstm2*		X			X			X
15	0.53	1.51E-03	*Srebf1*			X					
16	0.53	2.55E-02	*Slc27a1*						X		
17	0.51	2.56E-04	*Abcc3*		X			X	X		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	*Mafg*			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				

Data are from male C57BL/6 J mice fed HF plus WR vs. HF diet for 10 weeks. Abbreviations: AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; FXR, farnesyl X receptor; HF, high-fat diet; LXR, liver X receptor; Nrf2, nuclear factor erythroid 2–related factor 2; PPARα, peroxisome proliferator–activated receptor alpha; PPARγ, peroxisome proliferator–activated receptor gamma; PXR, pregnane X receptor; WR, watermelon rind.

Regarding most downregulated genes, all 3 watermelon-treated groups had a varied set of mRNAs related to various metabolic pathways. The WF/HF mRNAs identified include several genes related to sterol and xenobiotic metabolism. For WR/HF, some of the downregulated genes indicate a reduction in hepatic inflammation, including *Cxcl1* (-1.44-fold), *Inhbb* (-1.27-fold), and *S100a8* (-1.04-fold; Table 5). With WS/HF, the most downregulated genes are *Lars2* (-2.02-fold), *Dhrs9* (-1.42-fold), and *Scara5* (-1.37-fold; Table 6).

To identify those 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, PPARα, PPARγ, and PXR. Lists of mRNAs that are significantly regulated ($P < 0.05$) or that show 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; 22 of them are known to be regulated by PPARα, 13 are known to be regulated by CAR, and 10 are known to be regulated by AhR. For the WR/HF comparison (Table 8), there were fewer mRNAs associated with the list of transcription factors, with only PPARα having 15 mRNAs. For the case of WS/HF (Table 9), there were 22 mRNAs associated with PPARα, 10 associated with CAR, and 7 with PXR.

Finally, to focus on the transcription of those cytokine genes indicative of inflammation, we show 4 commonly measured pro-inflammatory mRNAs (CRP, TNF, IL1B, and *Ccl2*; **Figure 2**). Mice in the HF control group had significantly higher CRP mRNA levels, as compared with mice in the other treatments (Figure 2A). The dietary groups showed no significant differences regarding the expression of TNF and IL1B (Figure 2B and C). For the case of *Ccl2*, mice in the HF control group had greater mRNA levels than LF-fed mice, and the mice in the WR group had mRNA levels that were reduced to the point that they were statistically indistinguishable from mice in the LF group (Figure 2D).

Discussion

In our prior report (19), WF 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. The consumption of high-fiber WR (2.25% w/w) also improved glucose metabolism, serum insulin, and food efficiency, while WS (2.25% w/w) and WR both improved the microbiome composition. In the present study, we evaluated the impact of the inclusion of WF and fiber-rich WR and WS byproducts on hepatic gene expression. The goal of the present work was to identify mechanistic regulatory factors and pathways that are altered by our 3 test diet ingredients. There were

TABLE 9 Most significantly regulated hepatic mRNAs and associated ligand activated transcription factors from high-fat diets with and without watermelon skin

No	Log2 Fold Change	*P* Value	Gene Name	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			X
3	1.29	1.02E-10	*Cidec*							X	
4	1.13	2.47E-08	*Acot2*						X		
5	0.88	8.63E-05	*Ugt1a5*						X		X
6	0.79	2.49E-06	*Gstm1*		X			X	X		X
7	0.77	1.12E-03	*Cyp4a10*						X		
8	0.75	9.66E-04	*Gstm3*	X	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	*Acot4*						X		
12	0.67	3.23E-03	*Cidea*							X	
13	0.65	4.72E-03	*Cyp46a1*				X				
14	0.65	4.08E-03	*Slc27a1*						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	*Srebf1*			X					
21	0.49	8.47E-03	*Cyp4a14*						X		
22	0.48	4.21E-02	*Slco1a4*		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	*Abcc2*		X	X			X		
27	0.23	4.37E-02	*Slc2a2*							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	*Slco1a1*	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		

Data are from male C57BL/6 J mice fed HF plus WS vs. HF diet for 10 weeks. Abbreviations: AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; FXR, farnesyl X receptor; HF, high-fat diet; LXR, liver X receptor; Nrf2, nuclear factor erythroid 2–related factor 2; PPARα, peroxisome proliferator–activated receptor alpha; PPARγ, peroxisome proliferator–activated receptor gamma; PXR, pregnane X receptor; WS, watermelon skin.

a profound set of mRNAs differently regulated between mice in the LF and HF control groups. Briefly, upon evaluation of the most up- and downregulated canonical pathways, lipid metabolism was robustly upregulated, including in both fatty acid and sterol-related pathways. We suggest that the prominent activation of these pathways is consistent with the upregulation of both β-oxidation and alterations of bile acid metabolism.

When evaluating a collection of mRNAs regulated by ligand-dependent transcription factors, such as PPARα and PXR, we identified a very strong relationship between watermelon intake and PPARα regulation. This relationship alone would suggest that watermelon consumption delivers a dietary compound, or a compound biotransformed by the microbiome, that acts to agonize to PPARα-regulated gene transcription. Strongly supporting this hypothesis is the identification of *Cyp4a10* and *Cyp4a14* as the 2 most upregulated genes in the livers of mice in the WF group, as compared to mice in the HF control group, since the Cyp4a family of genes is known to be strongly upregulated by PPARα. The other mRNAs found to be most strongly upregulated with WF intake are *Acot3*, involved in the 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).

The relatively large number of mRNAs upregulated by the xenobiotic-sensing receptors CAR and PXR provides further evidence that bioactive compounds from watermelon impact liver functions. For example, 4 of the 6 most upregulated mRNAs seen in mice in the WS group are known to be regulated by PXR and/or CAR. The upregulation 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

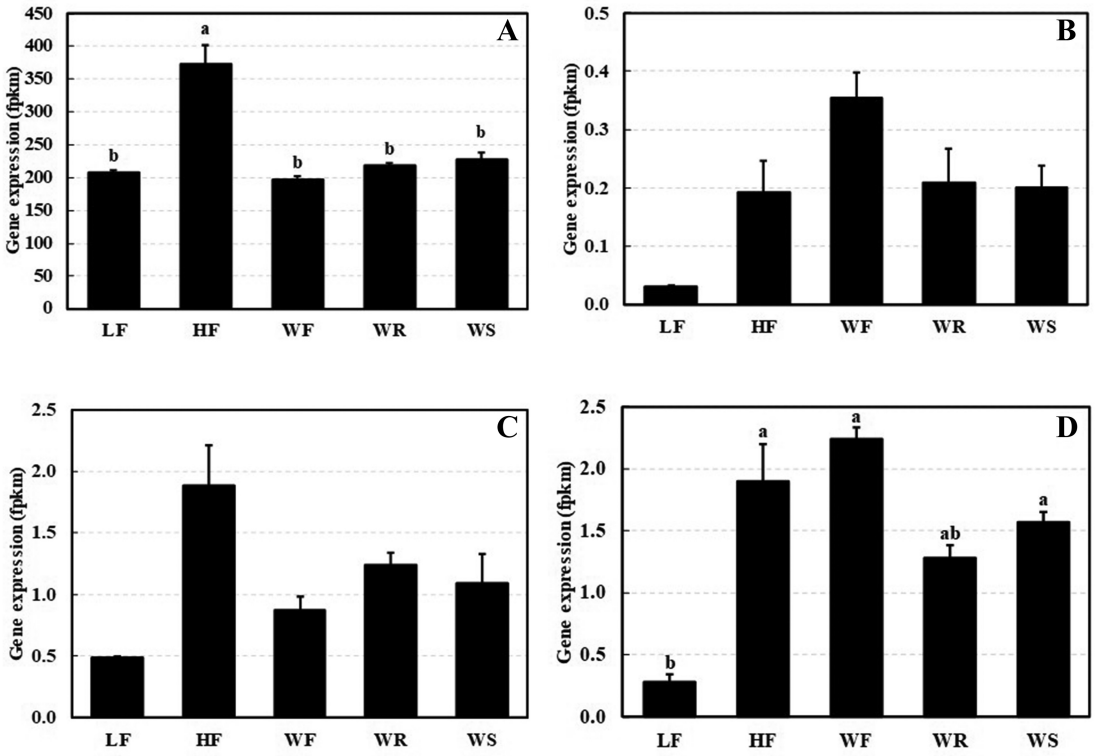

FIGURE 2 CRP, TNF, IL1B, and *Ccl2* cytokines expressed in fragments per kilobase per million reads (A, B, C, and D, respectively) of liver from male C57BL/6 J mice fed with LF, HF, HF plus WF, HF plus WR, and HF plus WS. Diets with the same letters have statistical significance when compared using the Tukey test ($P < 0.05$). Abbreviations: CRP, C-reactive protein; HF, high-fat diet; LF, low-fat diet; WF, watermelon flesh; WR, watermelon rind; WS, watermelon skin.

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 the fecal elimination of bile acids to determine whether there is any significant impact.

Also of note was the determination that circadian regulation and rhythm are the 2 most upregulated pathways associated with WR intake. Although they are not fully understood, circadian pathways are believed to be regulated, in part, by the retinoic acid–related orphan receptor (ROR), and there is likely some cross talk between circadian rhythms and lipid metabolism. A notable example is *Cyp7b1*: this mRNA is known to be downregulated 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 the circadian rhythm. A possible cause of increased *Per3* gene expression is the decrease in nonesterified fatty acids, as was previously reported by another study in our lab (19).

For the case of downregulated canonical pathways, nearly all 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 downregulated pathways. For the case of WR/HF, most of the top 20 pathways relate to downregulation of the unfolded protein response pathway (30, 31), with 3 other pathways related to inflammation and the immune response. This finding strengthens the conclusions of a prior study by Becraft et al. (19), which reported reduced levels of proinflammatory fatty acids in the liver, as determined by a metabolomic analysis.

Canonical pathways related to endoplasmic reticulum stress and the unfolded protein response (UPR) were robustly downregulated 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 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 (33–35). The unfolded protein response regulates 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, is a post-transcriptional regulator of cellular metabolism. The substantial downregulation of endoplasmic reticulum stress and UPR that was uniquely found in the WR group further supports the notion that constituents of WR are

impacting the regulation of the circadian rhythm, which may assist in the physiological stress response to increased adiposity.

For WS/HF, at least 8 other pathways were related to downregulation 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 metabolisms. 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, versus the HF control group, and are considered to be regulated by 1 of the ligand-regulated factors listed above. The most common factor in all 3 lists of genes was PPARα, suggesting these 3 diets all improve lipid metabolism via PPARα-induced changes in β-oxidation and other lipid catabolic processes. The consumption of WF and WS appeared to impact the xenobiotic-related factors (AhR, CAR, and PXR) more profoundly than did the consumption of 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 3 watermelon diets. Liver X receptors are master regulators of hepatobiliary reserve cholesterol transport, which is 1 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 proteins and the pro-lipogenic transcription factor *Srebp*-1c, as well as proteins involved in lipid remodeling, such as cholesteryl ester transfer protein, phospholipid transfer protein, and lipoprotein lipase. Our results showed a response consistent with LXR activation by all the watermelon products (Tables 2–4), compared to HF control diet (16, 39).

Post-prandial hepatic activation of PXR and CAR promotes the clearance of toxic dietary metabolites, drugs, and xenobiotics through Phases I, II, and III xenobiotic metabolism (16). CAR and PXR are activated by many different phytochemicals. In this study, these receptors were also activated by WF and WS and, to a lesser degree, WR. The mRNAs regulated by AhR and Nrf2 generally follow this same pattern. It is also possible that the activity of retinoid X receptor (RXR) may be regulated by 1 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 the 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-kB (40) and activator protein 1 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 3 watermelon diets. Many ligands have been presented as possible agonists of PPARα. The natural compounds proposed as activators for PPARα are long-chain fatty acids, including polyunsaturated fatty acids such as linoleic acid, linolenic acid, eicosapentaenoic acid, 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 nonlipid phytochemicals.

The 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 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); thus, these compounds may act as activators for PPARα.

Inflammation plays a key role in the development of atherosclerosis. An indicator of systemic inflammation is CRP levels (43). In our study, the expression of CRP in mice in the WF, WR, and WS groups was the same as in healthy mice in the LF group. Hong et al. (11) demonstrated decreased serum CRP concentrations in male Sprague-Dawley rats after 9 weeks of watermelon consumption. In their study, lower serum CRP levels were associated with upregulation of 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 that watermelon intake provides an anti-inflammatory effect.

Despite its lower fiber content, WF impacts hepatic gene expression patterns significantly, suggesting that another component besides fiber is bioactive. A 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 a high-fat diet (46).

Another component somewhat unique to watermelon is citrulline. It has been theorized that citrulline mainly impacts physiology through the nitric oxide pathway and vasodilation (9). It is unclear whether citrulline intake impacts gene expression to any significant degree.

In conclusion, all 3 watermelon-supplemented groups exhibited changes in gene expression patterns, compared to mice in the HF control group. Each of the 3 watermelon treatments had uniquely expressed genes when compared with the other watermelon treatments, with 322, 99, and 62 differentially expressed genes for the WF, WR, and WS groups, respectively. These findings indicate that while all 3 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. Overall, how each watermelon component acts in these common and more unique pathways can be clearly seen (**Figure 3**).

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. The utilization of watermelon byproducts as functional fiber

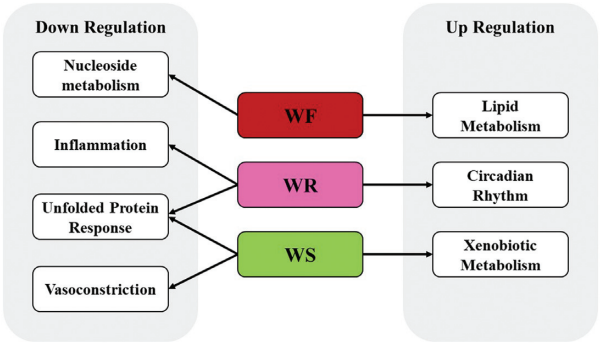

FIGURE 3 Selected most significant down- and upregulated hepatic canonical pathways in male C57BL/6 J mice fed HF plus WF, HF plus WR, and HF plus WS, compared to HF-fed mice. Abbreviations: HF, high-fat diet; WF, watermelon flesh; WR, watermelon rind; WS, watermelon skin.

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 WF 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 those observed in this study.

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