Original Research

Human skin gene expression: Natural (trans) resveratrol versus five resveratrol analogs for dermal applications

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Impact statement

Resveratrol has been reported to have a wide variety of health benefits but its rapid metabolism especially after oral ingestion results in very low bioavailability. Notably, the first human skin gene expression study of resveratrol was not published until 2014. The purpose of this study was to determine if increased stability and biological activity could be obtained by modifying the chemical structure of natural (trans) resveratrol and quantifying human gene expression by qPCR of skin biomarkers that enhance dermal health. Five resveratrol analogs were synthesized that increased their lipophilic index to enhance tissue penetration and augment biological activities on the measured parameters that expand the current knowledge of structure/function relationships. The butyrate and isobutyrate modifications displayed gene expression values significantly above resveratrol and suggest that oral application of these and potentially other resveratrol analogs may yield similar results to improve stability and biological activity to benefit/address various disorders/diseases.

Abstract

Resveratrol (RV) is a polyphenolic compound naturally produced by plants. Polyphenolic compounds incorporated into medicinal products are beneficial but, RV is rapidly metabolized with an associated decline in biological activity. This study tested RV as the standard and compared five structurally modified RV analogs: butyrate, isobutyrate, palmitoate, acetate, and diacetate (to improve functionality) at 1% concentration(s) for 24 h in epiderm full thickness cultures by gene array/qPCR mRNA analysis. When silent mating type information regulation 2 homolog 1, extracellular elements (collagen1A1, 3A1, 4A1; elastin, tissue inhibitor of matrix metalloproteinase 1, fibrillin 1 laminin beta1 and matrix metalloproteinase 9), anti-aging and aging genes, inflammatory biomarkers (interleukin-1A [IL1A], IL1R2, IL-6 and IL-8), nerve growth factor, and the antioxidants (proliferating cell nuclear antigen, catalase, superoxide dismutase and metallothionein 1H/2H) were evaluated, ranking each from highest-to-lowest for gene expression: butyrate > isobutyrate > diacetate > acetate > palmitoate. This study showed that the butyrate and isobutyrate analogs are more biologically active compared to resveratrol and have potential use in topical applications to improve dermal and other health applications.

Keywords: Resveratrol, analogs, human skin, gene expression, silent mating type information regulation 2 homolog 1, topical

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Introduction

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a polyphenolic compound naturally produced in abundance by several plants such as red grapes and Japanese knot weed.[1,2] Resveratrol (RV) is a known sirtuin activator that regulates many cellular activities which promote cell survival/delay or attenuate many age-related disorders, including its chemoprotective properties that were first reported in 1997 by John Pezzuto's laboratory.[3–5]

RV is also known for its antioxidant and anti-inflammatory activities.[2,4–6] Polyphenolic compounds in recent years have been incorporated into medicinal, over-the-counter (OTC), and cosmetic products. Notably, several authors have reported the protective action of RV in skin.[2,6–13] Also, our laboratory previously reported the influences of RV on human skin gene expression where it can ameliorate the aging of human skin by significantly stimulating SIRT1, extracellular matrix (ECM) proteins, such as collagens and elastin (ELN), and antioxidants while significantly inhibiting inflammatory and dermal-aging biomarkers.[4] However, as an effective natural compound in commercial products RV has been problematic due to its rapid metabolism, especially via oral dosing and possibly in cosmetics.[2,14] One recent report examined the RV analog, resveratryl triacetate

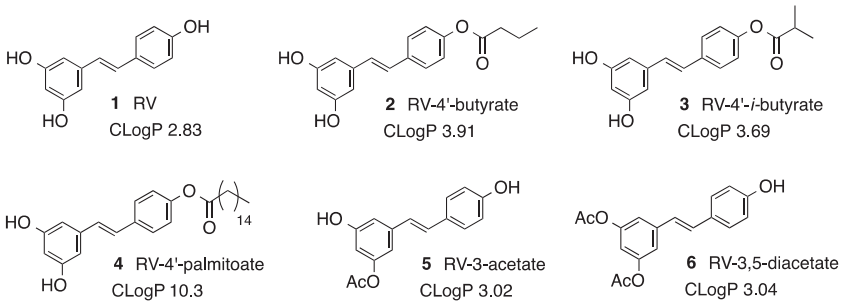

Figure 1 The chemical structures, chemical abbreviations, and hydrophilicity index (CLogP) are displayed for resveratrol and the five resveratrol analogs tested. 1: resveratrol (RV); 2: resveratrol-4′-butyrate (RV-4′-butyrate); 3: resveratrol-4′-isobutyrate (RV-4′-*i*-butyrate); 4: resveratrol-4′-palmitoate (RV-4′-palmitoate); 5: resveratrol-3-acetate (RV-3-acetate), and 6: resveratrol-3,5-diacetate (RV-3,5-diacetate). The ClogP values indicate how soluble each substance is in water; the higher the CLogP value the lower the solubility in water, or conversely, the higher solubility in lipid. Note: the five RV analogs will be referred to hereafter as: butyrate, isobutyrate, palmitoate, acetate, and diacetate

suggested that this RV analog, with increased stability, may be incorporated into cosmetic formulations that can whiten human skin without inducing skin irritation.[14] Other reports have demonstrated that: RV protects human keratinocytes from ultra-violet A-induced oxidative stress by downregulating Keap1 expression[15]; SIRT1 confers protection against ultra-violet B- and H_2O_2-induced cell death via p53 and c-Jun N-terminal kinase (JNK) in cultured skin keratinocytes[16] and, RV inhibits tumor necrosis factor (TNF)-alpha-induced proliferation and matrix metalloproteinase (MMP 9) expression by inhibiting nuclear factor (NF)-kappa B and activator protein-1 (AP-1) mechanisms.[17] More recently, our laboratory demonstrated that the RV analog, 4′-acetoxy RV (4AR) is more potent compared to RV when several biomarkers of human skin gene expression were examined.[18]

It is interesting to consider whether or not other analogs of RV may improve the functionality of synthetic ingredients in topical applications, nutraceutical supplements, and pharmaceuticals. Therefore, the purpose of this study was to examine five RV analogs: butyrate, isobutyrate, palmitoate, acetate, and diacetate and to compare the human skin gene expression results to natural RV using epidermal full thickness (EFT)-gene array/qPCR mRNA analysis. In general, for the 25 biomarkers quantified, when the gene expression was ranked from highest-to-lowest: butyrate > isobutyrate > diacetate > acetate > palmitoate when compared to the RV results. Thus, the results of the present study suggest that some RV analogs are more potent or biologically active compared to RV and have the potential to be used topically for the treatment of skin aging to improve human dermal health.

Materials and methods

Synthesis of RV analogs

The 4′-butyrate, isobutyrate, and 4′-acetate-RV analogs were produced in one step directly from RV using sodium hydride in dimethyl sulfoxide (DMSO) at 65°C with the corresponding acid anhydride. Isolated yields were moderate (40–47%) and the purity was high (>98%) following silica gel chromatography.[1] The 4′-palmitoate, 3-acetate, and the 3,5-diacetate RV analogs were produced in high purity (>98%) using a four-step route involving a decarbonylative palladium catalyzed Heck coupling with 4-acetoxystyrene and protected dihydroxybenzoyl chloride.[19]

As shown in Figure 1, the chemical structures, chemical abbreviations, and hydrophilicity index (CLogP) are displayed for RV and the five RV analogs. Note: the five RV analogs will be referred to hereafter as: butyrate, isobutyrate, palmitoate, acetate, and diacetate.

Control testing and validation (using untreated controls and DMSO as the control vehicle)

As reported in previous studies, multiple experiments were performed to evaluate the vehicle (DMSO controls; Sigma Chem. Co., St. Louis, MO, ACS reagent grade ≥ 99.9%) in short-term (24 h) incubations which had little influence on the measured parameters.[4,18] Untreated controls were used to validate the histological integrity of the skin sections (see below) and had no significant influence on the measured parameters.

Human epiderm full thickness (EFT)—gene array/qPCR-mRNA quantification

EFT skin cultures (MatTek, Ashland, MA) were employed that represent human skin barrier equivalents, which were reported elsewhere.[4,18,20,21] Each single well of the EFT cultures was visually inspected for physical imperfections and each had a surface area of 1.0 cm^2. The concentration of RV and the five analogs used was 1.0% (dissolved in 100% DMSO). A 20 μL sample was placed across each EFT skin culture by treatment group following published protocols.[18] EFT skin cultures ($n = 6$) were exposed for 24 h to 20 μL of: (1) 100% DMSO vehicle (control), (2) RV, and the RV analogs: (3) butyrate, (4) isobutyrate, (5) palmitoate, (6) acetate, and (7) diacetate. At the end of the 24-h incubations total RNA was isolated and quantified via qPCR analysis to determine human skin gene expression of 25 biomarkers (either significant stimulation or inhibition). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the control gene for all samples/experiments.[4] In brief, pilot studies revealed that 100% DMSO could be utilized as the skin penetrating agent via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

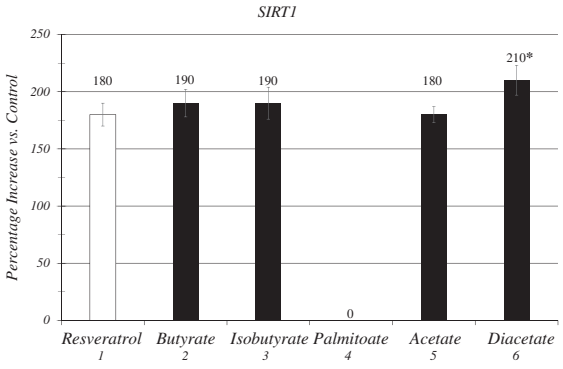

Figure 2 SIRT1 gene expression: topical application of resveratrol (RV) and the five RV analogs were tested in epiderm full thickness cultures by gene array/qPCR mRNA analysis where DMSO served as controls. The RV analog values were compared to the RV results. Only diacetate significantly stimulated SIRT1 gene expression to 210% or by approximately 17% over RV levels, while butyrate, isobutyrate, and acetate displayed levels similar to RV at 180%. Notably, palmitoate did not stimulate or inhibit SIRT1 gene expression at all. SIRT1: silent mating type information regulation 2 homolog 1; DMSO: dimethyl sulfoxide

(MTT) viability analysis (data not shown graphically) and untreated controls were used as reference standard for histological purposes only.[4,18]

Histological analysis

To validate the integrity of the EFT skin cultures after application of the various treatments, slides were prepared and stained with hematoxylin/eosin that revealed cellular structures and epidermal/dermal borders. All treatment slides displayed intact and healthy epidermal layers (stratum corneum and keratinocytes) and dermal (fibroblasts) components, which have been reported previously[4,18] (data not shown graphically).

Statistical analysis

The Limma unpaired *t*-test was used to detect significant changes in gene expression between the treatment and control (DMSO) groups ($P < 0.005$ expressed as the mean ± SEM). Inhibition of gene expression was detected by significantly lower copy numbers and significant stimulation of gene expression was detected by significantly higher copy numbers compared to control (DMSO) values for each biomarker.[4,18]

Results

Topical application of only diacetate significantly stimulated gene expression of SIRT1 over RV

When the five RV analogs were tested against RV for their effects on SIRT1, only diacetate significantly stimulated gene expression to 210% for this biomarker over RV levels at 180% (Figure 2). The other RV analogs: butyrate, isobutyrate, and acetate yielded SIRT1 levels similar to RV alone, while palmitoate did not alter SIRT1 levels at all (Figure 2).

Topical application of RV analogs on the collagens, ECM elements and collagen metabolizing enzymes

All of the RV analogs significantly stimulated collagen 1A1 (COL1A1) gene expression above RV at 225%, except acetate. Butyrate was the highest at 350% followed by isobutyrate, palmitoate, and then diacetate at 270% (Table 1). For collagen 3A1 (COL3A1), butyrate displayed the highest stimulation, followed by isobutyrate = palmitoate compared to RV at 230%. Diacetate was not significantly different compared to RV COL3A1 levels. Only isobutyrate stimulated collagen 4A1 (COL4A1) above RV levels at 160%. Finally, acetate did not alter COL3A1 or COL4A1 levels (Table 1).

For the ECM elements only butyrate, isobutyrate, and diacetate displayed consistent stimulation among the quantified biomarkers (Table 1). For example, butyrate and diacetate significantly stimulated ELN levels above RV values at 180%. Isobutyrate, palmitoate, and acetate did not alter ELN levels at all. Only, butyrate, isobutyrate, and diacetate stimulated tissue inhibitor of MMP 1 (TIMP1) above RV levels at 230% (Table 1). Acetate was not significantly different than RV values and the palmitoate level was zero. While RV did not alter fibrillin1 (FBN1) levels, butyrate, isobutyrate, palmitoate, and diacetate significantly stimulated FBN1 levels over RV values. Acetate was equal to RV in not altering FBN1 levels. Only, isobutyrate and palmitoate stimulated laminin beta1 (LAMB1) above RV; while butyrate, acetate, and diacetate did not alter LAMB1 levels (Table 1).

Finally, for the collagen metabolizing enzymes, butyrate was similar to RV at −170% for inhibiting MMP 1 while isobutyrate, palmitoate, acetate, and diacetate did not alter MMP1 values (Table 1). For MMP9, diacetate displayed the highest inhibition at −800%, followed by butyrate when compared to RV at −480%. Isobutyrate inhibition of MMP9 was similar to RV, while palmitoate and acetate were significantly below RV levels (Table 1).

Topical application of RV analogs on anti-aging/aging and the inflammation biomarkers

For the anti-aging biomarker, transforming growth factor beta1 (TGFB1) only butyrate displayed a significant increase over RV. RV and all other RV analogs did not alter this biomarker (Table 2). Also, for the transforming growth factor beta receptor2 (TGFB2), only isobutyrate and diacetate significantly increased expression above RV levels at 150%. All other RV analogs displayed TGFB2 levels similar to RV values.

In quantifying the aging biomarkers, S100 calcium-binding protein A8 (S100 A8) all of the RV analog values were similar to RV at −340% (Table 2). For S100 A9, only butyrate significantly inhibited this biomarker more compared to RV at −290%. Isobutyrate and diacetate levels were similar to RV for S100 A9, while palmitoate and acetate displayed values significantly below RV levels (Table 2). Finally, for tumor necrosis factor receptor 1A, (TNFRSF1), only diacetate was similar to RV at −160%, while all other RV analogs displayed levels significantly below RV for this aging biomarker (Table 2).

Table 1 Gene expression of collagens, extra-cellular matrix (ECM) elements and collagen metabolizing enzymes—comparing five resveratrol analogs to resveratrol

Gene symbol	Gene name	1 Resveratrol	2 Butyrate	3 Isobutyrate	4 Palmitoate	5 Acetate	6 Diacetate
Collagens							
COL1A1	Collagen 1A1	225 ± 13	350 ± 10▲	300 ± 8▲	280 ± 6▲	0■	270 ± 20▲
COL3A1	Collagen 3A1	230 ± 11	290 ± 15▲	280 ± 12▲	280 ± 8▲	0■	190 ± 32
COL4A1	Collagen 4A1	160 ± 11	160 ± 10	180 ± 15▲	170 ± 14	0■	160 ± 18
ECM elements							
ELN	Elastin	180 ± 8	230 ± 20▲	0■	0■	0■	200 ± 13▲
TIMP1	Tissue inhibitor of matrix Metalloproteinase1	230 ± 12	280 ± 6▲	280 ± 19▲	0	220 ± 14	300 ± 14▲
FBN1	Fibrillin1	0	160 ± 12▲	190 ± 11▲	140 ± 7▲	0	150 ± 15▲
LAMB1	Laminin Beta1	0	0	160 ± 15▲	210 ± 5▲	0	0
Collagen metabolizing enzymes							
MMP 1	Matrix Metallo-proteinase1	−170 ± 14	−160 ± 20	0■■	0■■	0■■	0■■
MMP 9	Matrix Metallo-proteinase9	−480 ± 48	−670 ± 66◆	−550 ± 57	180 ± 24■■	0■■	−800 ± 58◆

▲Significantly greater gene expression compared to resveratrol (RV) 1.
■ Significantly less gene expression compared to resveratrol (RV) 1.
■■ Significantly less inhibition of gene expression compared to resveratrol (RV) 1.
◆Significantly greater inhibition of gene expression compared to resveratrol (RV) 1.

Table 2 Gene expression of anti-aging/aging and inflammation—comparing five resveratrol analogs to resveratrol

Gene symbol	Gene name	1 Resveratrol	2 Butyrate	3 Isobutyrate	4 Palmitoate	5 Acetate	6 Diacetate
Anti-aging/Aging							
TGFB1	Transforming growth Factor beta1	0	140 ± 10▲	0	0	0	0
TGFBR2	Transforming growth Factor beta receptor2	150 ± 13	150 ± 15	200 ± 17▲	130 ± 8	150 ± 13	180 ± 13▲
S100 A8	S100 Calcium-binding protein A8	−340 ± 100	−460 ± 120	−340 ± 150	−210 ± 38	−210 ± 38	−280 ± 45
S100 A9	S100 calcium -binding protein A9	−290 ± 33	−450 ± 29◆	−290 ± 17	−190 ± 25■■	−150 ± 32■■	−280 ± 25
TNFRSF1A	tumor necrosis factor Receptor 1A	−160 ± 10	−130 ± 12■■	0■■	0■■	0■■	−140 ± 10
Inflammation							
IL1A	Interleukin 1 alpha	−2200 ± 100	−2900 ± 150◆	−1600 ± 140■■	−360 ± 95■■	−530 ± 55■■	−900 ± 110■■
IL1R2	Interleukin 1 receptor2	−590 ± 24	−710 ± 30◆	−550 ± 26	0■■	−370 ± 36■■	−350 ± 29■■
IL-6	Interleukin-6	−3200 ± 120	−3100 ± 100	−2400 ± 65■■	−220 ± 18■■	−710 ± 115■■	−1500 ± 130■■
IL-8	Interleukin-8	−790 ± 40	−950 ± 33◆	−600 ± 42■■	0■■	−190 ± 60■■	−280 ± 28■■

▲Significantly greater gene expression compared to resveratrol (RV) 1.
■■ Significantly less inhibition of gene expression compared to resveratrol (RV) 1.
◆Significantly greater inhibition of gene expression compared to resveratrol (RV) 1.

In examining the inflammatory biomarkers, only butyrate significantly inhibited levels more than RV for interleukin1 alpha (IL1A), interleukin 1 receptor 2 (IL1R2) and interleukin-8 (IL-8) (Table 2). For IL-6, butyrate showed a similar inhibition compared to that of RV at −3200%, while all other RV analogs displayed significantly less inhibition for these inflammatory biomarkers compared to RV alone (Table 2).

Topical application of RV analogs on nerve growth factor (NGF) and the antioxidants

Butyrate, isobutyrate, acetate, and diacetate significantly stimulated NGF levels above RV at 800%, while palmitoate did not alter this biomarker (Table 3). In general, acetate and diacetate had the most consistent stimulatory influence on the antioxidants: proliferating cell nuclear antigen (PCNA), catalase (CAT), superoxidase dismutase (SOD), and

Table 3 Gene expression of growth factor and antioxidants—comparing five resveratrol analogs to resveratrol

Gene Symbol	Gene Name	1 Resveratrol	2 Butyrate	3 Isobutyrate	4 Palmitoate	5 Acetate	6 Diacetate
Growth factor							
	Nerve growth factor (NGF)	800 ± 53	1400 ± 50▲	1300 ± 27▲	0■	900 ± 18▲	1600 ± 40▲
Antioxidants							
	PCNA proliferating cell						
	Nuclear antigen	780 ± 2	1010 ± 10▲	1000 ± 17▲	180 ± 21■	650 ± 11■	900 ± 20▲
	Catalase (CAT)	180 ± 23	210 ± 21	300 ± 6▲	170 ± 23	280 ± 20▲	270 ± 18▲
	Superoxide dismutase (SOD)	160 ± 10	180 ± 12	230 ± 9▲	0■	190 ± 8▲	190 ± 9▲
	Metallothionein 1H (MT1H)	4100 ± 20	6200 ± 18▲	4100 ± 16	0■	4900 ± 13▲	2600 ± 25■
	Metallothionein 2H (MT2H)	200 ± 8	230 ± 9▲	250 ± 21▲	0■	260 ± 14▲	270 ± 13▲

▲Significantly greater gene expression compared to resveratrol (RV) 1.
■ Significantly less gene expression compared to resveratrol (RV) 1.

metallothionein 1H & 2H (MT1H, MT2H) compared to RV values (Table 3). Following acetate and diacetate for the antioxidants, isobutyrate > butyrate while palmitoate had significantly less stimulatory influence on four out of the five biomarkers compared to RV values (Table 3).

Ranking of the RV analogs by gene biomarker compared to natural RV

Notably, SIRT1 was not included in the overall ranking schedule because diacetate was the only analog that displayed a significantly higher value, while palmitoate displayed the only significantly lower value compared to RV and the other three analogs (butyrate, isobutyrate and acetate) were not significantly different compared to RV.

The other ranking information is displayed in Figure 3, where the gene expressions of the biomarkers by table (gene expression category) were combined and the numerical values of each RV analog are shown in a tabular format. Note the analog with the lowest numerical value had the greater impact for a given biomarker (Figure 3). In brief, to determine the impact of the RV analogs on each biomarker quantified, the five RV analogs were ranked first through fifth when compared to the RV level. Thus, a ranking of first was assigned a numerical value = 1, while a ranking of fifth was assigned a numerical value = 5. Therefore, the RV analog with the lowest numerical value had the greatest impact on the gene biomarkers. For example, Table 1 results that covered the gene expression of the collagens, ECM elements and collagen metabolizing enzymes, the lowest numerical value was associated with butyrate, followed by isobutyrate, then palmitoate and diacetate with acetate having the largest numerical value (Figure 3).

For Table 2 results that covered the gene expression of anti-aging/aging and inflammatory biomarkers the lowest numerical value was associated with butyrate, followed by isobutyrate, diacetate, acetate, and then palmitoate displayed the largest numerical value (Figure 3).

When the gene expression of NGF and the antioxidants covered in Table 3 were analyzed, an interesting numerical ranking was obtained. Butyrate, isobutyrate, acetate, and diacetate values were low and similar to each other, while palmitoate displayed the highest numerical value. These data suggest that for these biomarkers four out of the five RV analogs had a comparable impact.

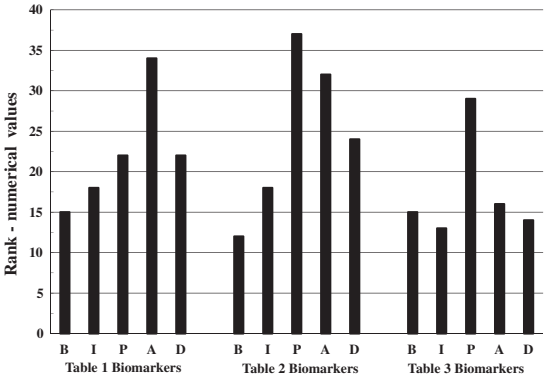

Figure 3 Ranking of the five RV analogs compared to the RV results by the biomarkers from Table 1, Table 2 or Table 3. In Table 1 there were nine biomarkers; Table 2, nine biomarkers, and Table 3, six biomarkers. To determine the impact of the RV analogs on each biomarker quantified, the five RV analogs were ranked 1st through 5th when compared to the RV level. Thus, a ranking of 1st was assigned a numerical value = 1, while a ranking of 5th was assigned a numerical value = 5. Therefore, the RV analog with the lowest numerical value had the greatest impact on the gene biomarkers. Finally, when the numerical values (by ranking) of the RV analogs were summed among all the gene biomarkers (including the SIRT1 results) then: butyrate = 44, isobutyrate = 51, diacetate = 61, acetate = 84, and palmitoate = 91. RV: resveratrol

Finally, when the numerical values (by ranking) of the RV analogs were summed among all the gene biomarkers then: butyrate = 44, isobutyrate = 51, diacetate = 61, acetate = 84, and palmitoate = 91. Thus, the impact of the RV analogs among all of the biomarkers tested can be ranked where butyrate > isobutyrate > diacetate > acetate > palmitoate. These data suggest that butyrate and isobutyrate may be the best RV analogs for incorporation into topical applications to improve dermal health among all of the analogs tested.

Discussion

RV is known for its anti-cancer, anti-inflammatory, and antioxidant properties.[2,4–6] However, the incorporation of RV

into commercial products present a challenge due to its rapid metabolism especially via oral applications. Thus, analogs of RV have been studied for increased functionality and biological activity as active ingredients in topical.[14,18] In the present study, five RV analogs were tested and based upon the CLogP values for each analog a prediction of increased solubility was somewhat reasonable to postulate. Although, the palmitoate analog with a CLogP value at 10.3 displayed the lowest impact on the human skin gene biomarkers tested; this was potentially due to its long methylene sidechain that may have caused conformational interference. The diacetate and acetate analogs displayed modest improvements in the quantified biomarkers with CLogP values just above RV (at 2.83), while butyrate and isobutyrate with CLogP values at 3.91 and 3.69, respectively, had the greatest positive impact on the skin-related biomarkers. The butyrate and isobutyrate analog results suggest that increased biological activity is associated with its ester chemical characteristics; where many examples are seen in other commercial products like vitamin C, aspirin, cocaine, and other compounds.[22,23]

Among the RV analogs, all stimulated SIRT1 human gene expression in a similar manner to RV except diacetate which improved SIRT1 levels by a significant 17% over RV at 180%. It has been reported that SIRT1 inhibits MMPs directly or indirectly that are known to break down collagen and ELN.[4,24]

The present results confirm and extend this concept where butyrate inhibited MMP1 at similar levels to RV and the diacetate and butyrate analogs significantly inhibited MMP9 gene expression to a greater degree compared to RV.[4,24] In support of the MMP results in the current study diacetate, butyrate, and isobutyrate all significantly stimulated TIMP1 levels over that of RV at 230%.

For the collagens, butyrate and isobutyrate displayed the greatest significant stimulation over RV levels, while for ELN only butyrate and diacetate showed significant stimulatory activity compared to RV values. It is well known that the collagens provide the important underlying scaffolding while ELN fibers are paramount in the elastic recoil properties in the dermal layer of youthful skin.[4,25,26]

A potential mechanism of how the SIRT1 activators stimulate TIMP1 and at the same time inhibit MMPs to enhance collage and ELN is shown in Figure 4. Also, polyphenolic/phytoestrogen compounds have been shown to increase collagen and ELN at the protein level, confirming the gene expression results in the present study.[4,20,21,27]

The gene expression of the anti-aging/aging and inflammatory biomarkers revealed that the butyrate analog displayed the greatest influence on these parameters. For example, TGFB1 is a known anti-aging factor that stimulates collagen synthesis and the aging biomarker S100 calcium-binding proteins (S100 A9) is known to increase with aging where it promotes inflammation in chronological and photo-aging.[18,25,28] Furthermore, almost all of the inflammatory biomarkers were significantly inhibited more with the butyrate analog compared to RV values, while all other RV analogs displayed values that inhibited the parameters to significantly less values compared to RV levels. This suggested that butyrate was the most effective analog to inhibit

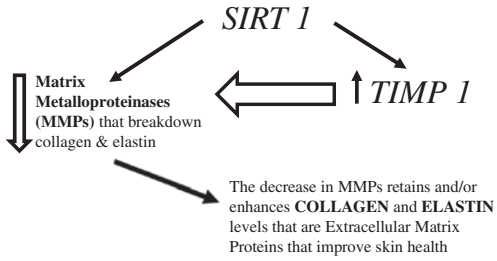

Figure 4 Potential mechanisms by which resveratrol (RV) and the RV analogs stimulate SIRT1 that directly or indirectly enhance collagen and elastin levels. This is accomplished by the stimulation of TIMP1 that in turn inhibits the MMPs which are known to breakdown collagen and elastin.
SIRT1: NAD-dependent deacetylase sirtuin-1 or silent mating type information regulation 2 homolog 1; TIMP1: tissue inhibitor of the matrix metalloproteinase 1; MMPs: matrix metalloproteinases

the inflammatory response that is known to increase reactive oxygen species (ROS) that in turn has a negative impact on collagen and ELN levels in the dermis.[18,27,29–32]

Surprisingly, for the NGF biomarker all of the RV analogs significantly stimulated gene expression over RV levels, except the palmitoate analog that did not alter NGF levels. NGF has been shown to stimulate tissue repair in human skin.[33] Moreover, this RV analog pattern was seen when the antioxidant biomarkers were tested. For example, all of the RV analogs, in general, significantly stimulated the antioxidant biomarkers while palmitoate displayed the least influence. These antioxidants play very important roles in preventing disease and maintaining dermal health.[18,27,34] Interestingly, it is important to recall that the skin has greater antioxidant activity compared to internal organs, since it is the first line of defense.[34] In this regard, the antioxidants: CAT, superoxide dismutase (SOD), and metallothioneins (MTH1 and MTH2) play vital roles in maintaining and enhancing human skin health by protecting against the harmful effects of free radicals and other toxic agents such as heavy metals.[27,35–38]

Since RV is metabolized rapidly in the body, it is important to test other analogs of RV in an attempt to improve the functionality of synthetic ingredients in topical applications. The present study examined five RV analogs where butyrate and isobutyrate appear to the best analogs to consider in future topical applications to enhance dermal health. Additionally, we studied the RV analog, 4′-acetoxy RV (4AR). Notably, 4AR significantly stimulated SIRT1 gene expression by 335% compared to RV at 180% and significantly altered many other skin biomarkers versus RV values.[18] When 4AR was included in the rankings/comparison for the quantified biomarkers among the RV analogs in this study from highest impact-to-lowest: 4AR > butyrate > isobutyrate > diacetate > acetate > palmitoate.[4,18] Thus, the addition of an acetoxy group to RV apparently has a greater impact on the human skin related-biomarkers compared to the addition of an ester for butyrate and isobutyrate. Additionally, four AR, as previously reported, are currently used in cosmetic applications, where it has a greater impact compared to RV.[4,18] For any of the RV analogs tested it is unlikely that they acted as prodrugs or that phase II metabolism occurred in skin (via conjugation pathways) to any

significant extent since this enzymatic activity only operates in the picomole range compared to the liver that has very high conjugation levels, which has been discussed elsewhere.[18,39]

It should be noted that phase I metabolism has been reported in intact human skin samples and comparisons have been made to *in vitro* skin models where, in general, the degree or levels of cytochrome 450 (CYP) and esterase activity are similar among the human epidermal/dermal layers and the 3D human skin models with epidermal components.[40–43] It is not known whether esterase activity via phase I metabolism played a role in the outcomes of the present study. However, phase II metabolism in intact skin and in *in vitro* skin models, in general, possess much lower conjugation and monooxygenation (via P450s) compared to liver tissue.[41] Finally, the use of DMSO has been used clinically in cardiac and central nervous system (CNS) applications and the penetration characteristics, presumably, are similar between intact human skin and the *in vitro* skin models.[44]

In summary, this study showed that the RV analogs: butyrate and isobutyrate can ameliorate the aging of human skin by significantly altering the gene expression of SIRT1, collagens, extra cellular matrix proteins (like elastin, fibrillin and laminin), while at the same time significantly inhibited the inflammatory and aging biomarkers. Other studies have investigated how RV analogs may improve dermal health.[14,18] However, further studies are underway in our research efforts to determine how chemical modifications of RV can positively impact dermal and other health applications.

Authors' contributions: EDL was involved with all phases of this study (providing funding support, experimental design, experimentation, data analysis and authorship of the manuscript); and MBA was involved in the chemical synthesis of the RV analogs, experimental design and authorship of the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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