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The ability of quercetin and ferulic acid to lower stored fat is dependent on the metabolic background of human adipocytes

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Running Title: Polyphenols reduce adipocyte lipid storage

ABBREVIATIONS

ACAT2	Acetyl-CoA acetyltransferase 2
ADH4	Alcohol dehydrogenase 4
ATP1A2	ATPase Na+/K+ transporting subunit alpha 2
CD36	Cluster of differentiation 36, fatty acid translocase
CE	Cholesteryl esters
CYP4B1	Cytochrome P450 B1
DAG	Diacylglycerols/diglycerides
DCER	Dihydroceramides
FASN	Fatty acid synthase
(F)FA	(Free) fatty acids
FKBP5	FK506 (tacrolimus) binding protein 5
Fr A	Ferulic acid
HCER	Hexosylceramides
HMGCR	3-hydroxy-3-methyl-glutaryl-CoA reductase
HSD17B6	Hydroxysteroid 17-beta dehydrogenase 6
IPA	Ingenuity pathway analysis
LPC	Lysophosphatidylcholines
LPL	Lipoprotein lipase
LXR	Liver X receptor
MAG	Monoacylglycerols/monoglycerides
MAP2K6	Mitogen-activated protein kinase kinase 6
MVD	Mevalonate diphosphate decarboxylase
NFATC2	Nuclear factor of activated T cells 2
ORO	Oil Red O
PC	Phosphatidylcholines
PE	Phosphatidylehanolamine
PPAR	Peroxisome proliferator-activated receptor
PRKAR2B	Protein kinase cAMP-dependent type II regulatory protein beta subunit
PTGER3	Prostaglandin E receptor 3
Q	Quercetin
RNA-seq	RNA sequencing
RXR	Retinoid X receptor

SEM   Standard error of the mean

SGBS   Simpson-Golabi-Behmel syndrome

T3   Triiodothyronine

TAG   Triacylglycerols/triglycerides

"Ongoing lipogenic state": differentiated human SGBS adipocytes containing stored lipids

under conditions where they are actively making and accumulating additional lipids

"Lipid storage state": differentiated human SGBS adipocytes containing stored lipids under

conditions where, in the absence of external modulators, they are maintaining but not

increasing stored lipids

1 **ABSTRACT**

2 **Scope**: Dietary flavonoids and phenolic acids can modulate biomarkers of lipid metabolism.

3 **Materials and methods**: Differentiated human SGBS adipocytes containing stored lipids,

4 mimicking white adipose tissue, were cultured either under conditions where they are

5 actively making and accumulating additional lipids through lipogenesis ("ongoing lipogenic

6 state") or under conditions of maintaining but not increasing stored lipids ("lipid storage

7 state"). The cells were assessed for total lipids, by lipidomic analysis, and by transcriptomics.

8 In the "lipid storage state", longer-term treatment with multiple low doses of quercetin,

9 ferulic acid or both together significantly reduced stored lipid content, modified genes

10 relating to lipid metabolism, with a strong implication of PPARα/RXRα, and altered lipid

11 composition. In the "ongoing lipogenic state", a higher concentration of quercetin was

12 required to attenuate stored lipid content, with no effect of ferulic acid, and there were fewer

13 changes in gene expression, no detectable involvement of PPARα/RXRα, and fewer changes

14 in lipid composition.

15 **Conclusions**: Chronic low-dose treatment of quercetin and ferulic acid modulates lipid

16 metabolism in adipocytes, but the effect is dramatically dependent on the metabolic state of

17 the cell.

INTRODUCTION

The utilisation of stored lipids from white adipose tissue is a vital part of deriving energy for human activity (1), but excessive storage of triglycerides is related to increased risk of adverse cardiovascular events and developing type 2 diabetes (2). Flavonoids such as quercetin protect against metabolic dysfunction in rodent high-fat diet models (3-5), and against diabetic symptoms in streptozotocin-treated rats (6). The phenolic acid, ferulic acid, can reduce the body weight of high fat diet-fed mice (7, 8). The effects of quercetin and ferulic acid on adipocytes may be partially mediated via the gut microbiota (4) and via hormonal and inflammatory processes (7), although key signalling pathways within adipocytes remain to be elucidated.

In vitro cellular studies using murine-derived cell lines have shown that incubation with high concentrations of (poly)phenols (which include flavonoids and phenolic acids, for nomenclature see Frank et al 2019 (9)) can slow, or even prevent, the differentiation of pre-adipocytes towards functionally mature lipid-storing cells (10-13). Triacylglycerol (TAG) content was lower in murine 3T3-L1 adipocytes after several doses of quercetin during differentiation, but no further mechanistic experiments were performed (13). Quercetin has been proposed to directly affect mitochondrial processes, after significantly changing the expression of adipokine- and glycolysis-related genes in human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes (14), and was shown to dose-dependently attenuate tumor necrosis factor α (TNFα)-mediated inflammation in 3T3-L1 adipocytes (15). Oral administration of ferulic acid to rodents on a high-fat diet significantly reduced expression of fetuin-A, involved in lipid-induced adipocyte inflammation, and pro-inflammatory cytokines in adipose and circulation (7, 15). Ferulic acid at high concentration also attenuated 3T3-L1

42   adipogenesis via heme oxygenase-1 regulation (16). Crucially, the chronic effect and

43   mechanism of action on differentiated adipocytes remains unknown.

44

45   We report here the effects of repeated treatments over 3 d of low concentrations of quercetin

46   and/or ferulic acid on matured human SGBS adipocytes under conditions where they are

47   synthesising lipid, as a model for the effect on lipogenesis, or storing lipid, where the process

48   of fat utilisation, predominantly through fatty acid β-oxidation, becomes predominant.

MATERIALS AND METHODS

Materials

Preadipocyte cells of the Simpson-Golabi-Behmel syndrome (SGBS) strain originate from adipose tissue of a young patient with SGBS. These cells have been previously characterised (17-19). All reagents were purchased from Sigma-Aldrich, UK, unless otherwise stated, and were of the highest analytical grade available. A solution of 3.3 mM pantothenate with 1.7 mM biotin (termed pan-bio) was prepared in DMEM/F-12 medium (25 mM glucose) (Gibco, UK). Quercetin (Extrasynthese, France) and ferulic acid (Sigma-Aldrich) were prepared as master stock solutions of 50 mM in DMSO and stored in small aliquots at -20°C until required. A solution of 3% (w/v) Oil Red O was prepared using PBS and was stored at 4°C for up to 12 months.

Culture of SGBS cells

SGBS cells of passages 31-41 were used in this study and were routinely cultured in T175 tissue culture vessels (Corning, UK). Cell proliferation was promoted by culturing under DMEM/F-12 medium (25 mM glucose) supplemented with 1% (v/v) penicillin-streptomycin solution, 1% (v/v) pan-bio solution (to comprise basal medium) and 10% (v/v) fetal bovine serum (source: South America, Gibco, UK) (to comprise growth medium). Cells were incubated in a humidified atmosphere of 37°C and 5% CO_2, were routinely cultured every 3-4 d and passaged with trypsin. Pre-adipocyte SGBS cells were promoted to differentiate as previously described (18). In summary, SGBS cells were plated at a density of 3×10^3 cells cm^{-2} and incubated in growth medium for 4 d. Differentiation was initiated by incubation in basal medium supplemented with 125 nM transferrin, 20 nM human insulin, 100 nM cortisol, 0.2 nM triiodothyronine (T3), 25 nM dexamethasone, 250 µM 3-isobutyl-1-methylxanthin (IBMX) and 2 µM rosiglitazone (Cayman Chemicals, Cambridge Bioscience, UK)

7

74 (adipogenic medium) for 4 d. Subsequent lipogenesis was promoted by incubation in basal

75 medium supplemented with only transferrin, insulin, cortisol and T3 for 10 d, with the

76 medium being newly refreshed twice (**Fig. 1**). Cultured cells were visualised using a Leica

77 MD IL LED inverted brightfield microscope (Leica Microsystems, Germany) and imaged

78 with LAS X software (Leica Microsystems).

79

80 **Cellular uptake of radiolabelled 2-deoxy-D-glucose**

81 SGBS cells were cultured on 12-well tissue culture plates (Corning) and promoted to

82 differentiate for a total of 14 d as described above. Cells were incubated in DMEM/F-12 for 4

83 h without insulin before incubation with 0.55 mM deoxy-D-glucose (0.27 mCi/ml 2-[1-

84 ^{14}C(U)]-deoxy-D-glucose (PerkinElmer, UK)) in DMEM/F-12 (with no glucose) with vehicle

85 (DMSO) or 20 nM insulin for 30 min. Cells were then washed twice in DMEM/F-12 (with no

86 glucose), lysed in 1M NaOH and then neutralised with 1M HCl. Treatments were performed

87 in duplicate wells and pooled. Isotope activity was determined by liquid scintillation counting

88 (Tri-Carb 1900 TR Liquid Scintillation Analyzer, Canberra Packard, UK). Obtained counts

89 per min were corrected for total protein, measured in aliquots of the lysates using the

90 Bradford assay (Pierce Bradford Protein Assay Kit, Thermo Fisher Scientific, UK).

91

92 **Treatment of SGBS cells with (poly)phenols**

93 Maturing SGBS cells (14 d culture) were treated with vehicle (DMSO), quercetin or ferulic

94 acid or a combined quercetin and ferulic acid treatment at time zero and every 24 h for up to

95 48 h (Time = 0, 24 and 48 h) as shown in **Fig. 1**. Treatments were applied as additions to

96 basal media (cells in "lipid storage state") or to lipogenic media (ongoing lipogenesis).

97

98 **Detection of lipid accumulation by Oil Red O staining**

99   SGBS cells were cultured on 12-well plates, and following treatment, the medium was

100   removed and cells washed with warmed PBS before being incubated at room temperature in

101   4% paraformaldehyde for 30 min. Subsequently the fixative solution was removed and the

102   cells washed twice in PBS before being stored under PBS at $4^{o}C$ until required. PBS was

103   removed and fixed cells were immersed for 30 min in an Oil Red O (ORO) staining solution,

104   prepared by diluting 3:2 stock solution to milliQ water (Merck-Millipore, UK). The cells

105   were subsequently washed twice in water and once in PBS, then air-dried at room

106   temperature. The stain was solubilised by covering the monolayer in isopropanol for 5 min.

107   Samples from each well were collected and diluted 4-fold in isopropanol with a 100 µL

108   aliquot transferred to a clear bottomed 96-well plate (Corning). The absorbance of each

109   sample between 440 and 600 nm was measured using a PHERAStar FS plate reader (BMG

110   LabTech, Germany) with maximal absorbance at 514 nm used for analysis.

111

112   **Detection of free glycerol**

113   SGBS were plated, differentiated and treated as described above. On the third treatment day

114   at T = 72 h, 24 h after the final treatment, conditioned media was collected. The amount of

115   free glycerol was determined using a cell-based assay kit (Cayman Chemicals) according to

116   the manufacturer's protocol. Samples and standards were assayed using technical duplicates

117   in a 96-well plate (Nunc, Thermo Fisher Scientific) and absorbance at 540 nm was

118   determined by PHERAStar FS plate reader. The amount of free glycerol in treated samples

119   was calculated by subtracting mean A_{540} values of control with no glycerol with glycerol

120   reagent free controls (media only) from the mean value of the treatment, relative to a standard

121   curve.

122

123   **Determination of lipid species by mass spectrometry**

124   Cells were propagated in T75 tissue culture flasks and treated as shown in **Figure 1**. Four

125   flasks received each treatment and were subsequently pooled as a single sample. All samples

126   were collected at 72 h, 24 h after the final treatment. Cells in each flask were washed with

127   warmed PBS and were liberated by trypsin (5 min, 37°C, 5% CO_2). An aliquot (10 µL) was

128   collected and live cell number determined by automatic cell count (TC20, Bio-Rad, UK)

129   using trypan blue. Cells were pelleted from suspension by centrifugation at $200 \times g$ for 5 min,

130   and were promptly snap frozen in liquid nitrogen before being stored at -80°C. Cell pellets

131   were resuspended in deionised water and homogenised by ultrasonication. A small aliquot of

132   homogenate was removed and used to determine total protein amount by Bradford assay for

133   subsequent normalisation of lipid species. The remainder of each homogenate was subjected

134   to a modified Bligh-Dyer extraction with extracts being dried under nitrogen and

135   reconstituted in ammonium acetate dichloromethane:methanol. The extracts were transferred

136   to vials for infusion LC-MS/MS (liquid chromatography/mass spectrometry), performed on a

137   Shimadzu LC with nanopolyetheretherketone tubing and the SCIEX SelexIon-5500 triple

138   quadrupole linear ion trap (QTRAP). The samples were analysed via both positive and

139   negative mode electrospray. The 5500 QTRAP was operated in multiple reaction monitoring

140   (MRM) mode with a total of more than 1,100 MRMs (Metabolon, Potsdam,

141   Germany). Individual lipid species were quantified by taking the ratio of the signal intensity

142   of each target compound to that of its assigned internal standard, then multiplying by the

143   concentration of internal standard added to the sample. Lipid class concentrations

144   were calculated from the sum of all molecular species within a class, and fatty acid

145   compositions were determined by calculating the proportion of each class comprised by

146   individual fatty acids. Concentrations are expressed as nmol of lipid per mg protein in the

147   sample.

148

Transcriptomics analysis

Cells were treated as described above and detailed in **Figure 1**, but all samples were collected

at T = 54 h. The wells were washed with PBS and lysed with Lysis/Binding Solution

(Invitrogen, Thermo Fisher Scientific). Three wells per treatment were combined for each

sample and samples were stored at -80°C until required. A volume of 100% ethanol equal to

the collected volume was added to each sample and total RNA was extracted using an Aurum

Total RNA mini kit (Bio-Rad) as per the manufacturer's instructions. Isolated nucleic acids

were quantified by Nanodrop (ND-1000, LabTech International, UK), with all analysed

samples exhibiting a A_{260}/A_{280} ratio exceeding 1.9, and stored at -80°C. Total RNA (100 ng)

was used to generate Illumina-compatible stranded, polyA selected, RNA-seq (RNA-

sequencing) libraries using the TruSeq Stranded Total RNA Library Kit (Illumina, USA). The

resultant libraries were quantified on the TapeStation automated electrophoresis tool (Agilent

Technologies, USA) and Qubit fluorometer (Thermo Fisher Scientific) before pooling to

create an equimolar pool that was sequenced on an Illumina NextSeq 75 bp single end read

lane. Adaptor sequences and low-quality base calls were removed using Cutadapt software

(20) and the quality of the data determined using FastQC tool (Babraham Bioinformatics,

Babraham Institute, UK). The data was aligned to the human genome (hg38) using STAR,

the ultra-fast universal RNA-seq aligner (21), with reference to the RefSeq gene annotation

database (22), which was obtained from the University of California, Santa Cruz, Genome

Browser website (23). Binary alignment map (BAM) files containing the aligned data ordered

by genomic position where indexed using the SAMtools sequence alignment/map tool

program suite (24) and PCR duplicates were marked using the Picard genome analysis toolkit

(25). The aligned data quality was then determined using the Qualimap software (26). As

there were no issues with DNA contamination or PCR duplicates, the Rsubread software

package (27) was used to count reads mapping to each RefSeq transcript. Differentially

174 expressed transcripts were identified using the R package DeSeq2 (28). Transcripts with a p

175 value, adjusted for multiple testing, of less than 0.05 were identified as differentially

176 expressed.

177

178 **Analyses and statistical approaches**

179 Quantification of lipid deposition was carried out on 96-well plates, run in triplicate at 514

180 nm using isopropanol as a dilution control. The effect of (poly)phenol treatments was

181 assessed by the students' t-test for each treatment against vehicle treatment with level of

182 significance set at $p < 0.05$. Analysis and presentation of MS data was performed using the

183 Metabolon Surveyor^{TM} online tool. Pathway and gene analysis from the transcriptomics data

184 was performed using the Qiagen Ingenuity Pathway Analysis (IPA) tool (QIAGEN Inc.,

185 Netherlands (29)). The algorithms developed for IPA are detailed by Krämer and colleagues

186 (30). The relevance of gene expression to a pathway or disease was scored by Fishers Exact

187 Test p-value. A level of significance of $p < 0.001$ was set for the relationship of pathways to

188 diseases and metabolic functions. Data are presented as mean values ± the standard error of

189 the mean (SEM).

RESULTS

Establishing conditions of lipid storage and ongoing lipogenesis

Promoting differentiation of SGBS cells induced a change in morphology from day 4 with

some intracellular lipid deposits visible by microscopy from day 8 (**Fig. 2A**), clearly evident

after 14 d, and further increasing to day 17 (**Fig. 2B**). The storage of neutral intracellular lipid

was confirmed by Oil Red O staining (**Fig. 2B**). SGBS cells displayed a significant insulin-

sensitive component of 2-[1-^{14}C(U)]-deoxy-D-glucose uptake (**Fig. 2C**). From day 14 to 17,

there was a significant increase in the amount of lipid present in the cells under continued

presence of the differentiation medium ("ongoing lipogenic state"), but not if the cells were

cultured in basal media between days 14 and 17, when the intracellular stored lipid remained

constant ("lipid storage state") (**Fig. 2D**).

Cells at days 14-17 were used for experiments, in either the "ongoing lipogenic state" or

"lipid storage state", during which they were treated with one or more doses of quercetin,

ferulic acid or both, and compared to vehicle control. Cell number (**Fig. 3A**) and viability

(**Fig. 3B**) were not affected over this time by 3 repeated 24 h treatments of 1 µM quercetin, 1

µM ferulic acid or both compounds combined. For lipidomic analysis, 5 biological replicates,

with each derived from 4 pooled T75 flasks, were analysed. The time-point analysed was 72

h, i.e. 24 h after the final treatment (**Fig. 1**). For transcriptome analysis, 4 replicates were

initially performed but only 3 met the quality criteria; these 3 samples were subsequently

analysed and showed limited batch variation (**Fig. 4A**). The time-point analysed was 54 h, i.e.

6 h after the final treatment (**Fig. 1**). Whether a transcript was significantly altered by

treatment was independent of the level of expression of that transcript (**Fig. 4B**). Principal

component (PC) analysis (PCA) showed that 2 factors could clearly separate the dataset for

each treatment and account for ≥95% of the full variation. Principal component 1 contributed

215   very strongly to the separation (**Fig. 4C**) and all treatments represented a minimum of 85% of

216   variance.

217

218   **Comparison of untreated SGBS cells in the "lipid storage state" to "ongoing lipogenic**

219   **state"**

220   When cells in the "ongoing lipogenic state" were compared to those in the "lipid storage

221   state" at 54 h, 422 transcripts were significantly upregulated and 388 were downregulated.

222   The most highly upregulated transcripts included CYP4B1 (cytochrome P450 B1) ($\log_{10}$ ratio

223   = 4.51), ATP1A2 (ATPase Na^+/K^+ transporting subunit alpha 2) (4.25), FKBP5 (FK506

224   binding protein 5) (3.21), MAP2K6 (mitogen-activated protein kinase kinase 6) (2.92) and

225   MVD (mevalonate diphosphate decarboxylase) (2.34), and the most highly downregulated

226   transcripts included HSD17B6 (hydroxysteroid 17-β dehydrogenase 6) (-2.52), SLC7A14

227   (solute carrier family 7, member 14) (-2.31) and transcription factor POU2F2 (POU (Pit-Oct-

228   Unc) class 2 homeobox 2) (-2.26). Lipidomic analysis revealed a total of 1047 intracellular

229   lipid species, and showed that cells in an "ongoing lipogenic state" exhibited some

230   differences in lipid profile after 72 h compared to cells in the "lipid storage state". The former

231   showed an almost 6-fold decrease in the proportion of monoacylglycerol MAG (22:0),

232   together with some increases in the proportions of various di- and triacylglycerol species

233   (**Fig. 5**). For total lipid classes, only the hexosylceramides (HCER) were significantly

234   different (31.4%; $p = 0.0369$).

235

236   **Effects of treatment of SGBS adipocytes in a "lipid storage state"**

237   The treatment of 3 × 24 h repeated doses of 1 µM quercetin, 1 µM ferulic acid or both

238   together over 3 d attenuated ORO-stained intracellular lipid content. The combination was

239   stronger than quercetin treatment alone ($p < 0.05$) or ferulic acid alone (trend, $p = 0.075$)

14

240 (**Fig. 6A**). Treatment with the same concentration for 2×24 h doses (**Fig. 6B**) or with a

241 single dose over 72 h (**Fig. 6C**) showed no significant effect on ORO-stained lipid content.

242 Media collected from 3×24 h treatments of 1 μM quercetin at the end of the 72-h incubation

243 period contained a significantly higher level of free glycerol compared to vehicle-treated

244 control cells (**Fig. 6D**).

245

246 The changes were examined further by mass spectrometry to quantify individual lipid

247 species. Treatment for 3×24 h doses of 1 μM quercetin, 1 μM ferulic acid, or both

248 combined, did not significantly affect the absolute concentration of any total lipid class, but

249 changed the lipid composition as indicated by the mole fraction of each class (**Table 1**). The

250 combined treatment produced more changes overall than the compounds individually. The

251 most notable effect seen by quercetin alone was a 75% reduction in the MAG (22:0) fraction

252 ($p < 0.01$), and with ferulic acid alone there was a 64% increase in the free fatty acids fraction

253 ($p < 0.05$). When combined, ferulic acid only mildly alleviated the effect of quercetin on

254 MAG (22:0) while quercetin completely annulled the ferulic acid-induced increase in the

255 proportion of free fatty acids (**Table 1**).

256

257 The effect of 3×24 h treatments on cells under "lipid storage conditions" on the number of

258 gene expression changes is summarised in **Table 2**. 71.3% of genes altered by quercetin

259 treatment were also affected by ferulic acid, and 92.8% of genes altered by ferulic acid were

260 also changed by the quercetin treatment. For the combined quercetin and ferulic acid

261 treatment, a proportion (41.3%) of genes were solely altered compared to the individual

262 treatments (**Fig. 7A**). When gene expression changes were analysed by ingenuity pathway

263 analysis (IPA), the general category of lipid metabolism exhibited the strongest association

264 for quercetin ($p = 8.74 \times 10^{-18}$) and for ferulic acid ($p = 1.98 \times 10^{-14}$). Combined treatment in

265   lipid storage conditions was also highly significantly associated with fatty acid metabolism (p

266     $= 3.20 \times 10^{-14}$), and all treatments were strongly significantly related to multiple key

267   pathways and systems associated with lipid handling and development of obesity (**Fig. 7B**).

268   The effects of gene changes associated with quercetin treatment in lipid storage conditions

269   are predicted to significantly decrease the synthesis, accumulation and concentration of lipid

270   (**Fig. 7C**) (activation z-scores of -0.629, -0.889 and -2.370 respectively). The transcription

271   factor NFATC2 (nuclear factor of activated T cells 2) was also strongly significantly

272   increased by quercetin treatment. Expression changes due to ferulic acid treatment are

273   predicted to weakly increase synthesis of lipid (z-score 0.310) but to significantly reduce

274   concentration and accumulation of lipid (-1.468 and -0.635 respectively). Genes contributing

275   to these effects are displayed in **Fig. 7D**. Expression changes due to combined quercetin and

276   ferulic acid treatment are predicted to weakly increase synthesis of lipid (z-score 0.239) but

277   to significantly reduce concentration and accumulation of lipid (-1.406 and -0.941

278   respectively) (**Fig. 7E**). Notable gene changes, which are significantly associated with altered

279   lipid synthesis and/or storage with all treatments, are summarised in **Table 3**. Pathway

280   analysis of gene expression changes showed that the effects on lipid metabolism could be

281   linked to PPARα/RXRα (peroxisome proliferator-activated receptor-α/retinoid receptor X-α)

282   signalling, inducing a predicted downregulation of lipid metabolism when treated with 1 µM

283   quercetin (z-score = -2.111; $p = 4.8 \times 10^{-5}$) (**Fig. 7F**), 1 µM ferulic acid (z-score = -1.00; $p =$

284   1.14×10^{-3}) (**Fig. 7G**) or both (z-score = -1.155; $p = 4.06 \times 10^{-4}$) (**Fig. 7H**).

285

286   **Effects of treatment of SGBS adipocytes during ongoing lipogenesis**

287   Treatment of SGBS with 3×24 h repeated doses of 1 µM quercetin, 1 µM ferulic acid or

288   both showed no significant effect on ORO-stained neutral lipid accumulation under "ongoing

289   lipogenic conditions", and only a 10-fold higher concentration of combined ferulic acid and

290   quercetin was effective (**Fig. 8A and B**). Lipids were analysed after 3 × 24 h repeated doses

291   of 1 µM quercetin, 1 µM ferulic acid or both, and in accord there was no significant effect on

292   the absolute concentration of any total lipid class (**Table 4**). However, all treatments

293   generally lowered the free fatty acid fractions, which was not observed in the "lipid storage

294   state". Other notable changes in lipid composition include a shift to more unsaturated

295   triacylglycerol species, and an increase in ceramides, only with the individual quercetin and

296   ferulic acid treatments. Ferulic acid alone also induced a 5-fold increase in the PE (O-

297   16:0/18:2) fraction. The combined treatment lowered the relative saturated and mono-

298   unsaturated species, particularly the diacylglycerols, while there was a relatively large

299   reduction in the PC (18:0/14:1), seen only with the combination (**Table 4**).

300

301   For ferulic acid, 3 × 24 h repeated doses caused few changes in gene expression, but 3 × 24 h

302   repeated doses of 1 µM quercetin, and combined treatment, brought about more changes

303   (**Table 2**). 58.1% of genes altered by quercetin treatment were also altered in the combined

304   treatment (**Fig. 9A**). Lipid metabolism, small molecule biochemistry and metabolic disease

305   were notable modified functions, although the effect of ferulic acid treatment alone was small

306   (**Fig. 9B**). The effects of gene changes associated with quercetin treatment are predicted to

307   significantly decrease synthesis, conversion and accumulation of lipid (**Fig. 9C**) (activation z-

308   scores of -0.218, -0.453 and -0.264 respectively). Expression changes due to ferulic acid

309   treatment are predicted to significantly decrease conversion of lipid (-0.594), related to the

310   significantly reduced expression of ADH4 (alcohol dehydrogenase 4), HMGCR (3-hydroxy-

311   3-methyl-glutaryl-CoA reductase) and PTGER3 (prostaglandin E receptor 3). Other lipid

312   handling functions were not significantly affected by chronic 1 µM ferulic acid treatment.

313   Expression changes due to combined quercetin and ferulic acid treatment are predicted to

314   significantly decrease conversion of lipid (z-score -0.60), accumulation of lipid (z-score -

315 1.109) and concentration of lipid (z-score -1.328) (**Fig. 9D**). Notable gene changes in all

316 treatments that are significantly associated with altered lipid synthesis and/or storage are

317 summarised in **Table 5**.

318

319 Unlike in the "lipid storage state", changes in gene expression in cells in an "ongoing

320 lipogenic state" did not significantly associate with the PPARα/RXRα signalling canonical

321 pathway. Instead, the effects of quercetin and of combined quercetin and ferulic acid

322 treatments were most notably on the super-pathway of cholesterol biosynthesis (predicted

323 deactivation, z-score = -2.236, $P = 1.7 \times 10^{-7}$) and (-2.828, P = 2.44×10^{-10}) respectively.

324 Reduced cholesterol biosynthesis activity was associated with significantly reduced

325 expression of ACAT2 (acetyl-CoA acetyltransferase 2, experimental log ratio = -0.797),

326 HMGCS1 (3-hydroxy-3-methyl-glutaryl-CoA synthase 1, -0.736) and HMGCR (-1.072) in

327 chronic quercetin-treated cells, and with ACAT2 (-0.716), HMGCR (-0.962), MSMO1

328 (methylsterol monooxygenase 1, -0.871) and NSDHL (NAD(P)-dependent steroid

329 dehydrogenase-like, -0.684) with combined treatment.

DISCUSSION

Differentiated SGBS cells behave as functional human adipocytes (17, 18) with a white

adipose tissue-like phenotype (19). By following a differentiation protocol in high glucose

(18), SGBS cells time-dependently synthesise and accumulate lipid. The cells were then

maintained under conditions where they continued to carry out lipogenesis and increased

their intracellular lipid, or under conditions where they maintained the stored lipid but did not

increase the content. In order for a treatment to lower the amount of stored lipid under "lipid

storage state" conditions, the process of fat utilisation, mostly through β-oxidation, would

need to be stimulated.

In the "ongoing lipogenic state", compared to the "lipid storage state", there was a substantial

decrease in the proportion of monoacylglycerol (22:0) species, with increases in various

DAG and TAG species, expected to be formed for adipocyte differentiation and fat storage

(31). An increase in the proportion of the hexosylceramide class is indicative of cell

membrane synthesis, while levels of hexosylceramides in adipose and liver are also linked to

metabolic syndrome, notably insulin resistance, oxidative stress and inflammation (32-34).

Upregulation of CYP4B1, ATP1A2, FKBP5, MAP2K6 and MVD transcripts are indicative

of the ongoing lipogenesis and relate to obesity development in vivo. CYP4B1 is a target of

PPAR activation (35) and is associated with increased lipid synthesis and adipose tissue

expansion, with substantial upregulation in obese pigs (36). Increased ATP1A2 indicates

plasma membrane increases and tissue expansion, and has been linked to hyperphagia in mice

(37). FKBP5 expression in human adipose is increased in T2D patients, relating to insulin

resistance and hyperglycaemia, and may be a key factor in glucocorticoid-induced insulin

resistance (38). MAP2K6 activates p38 MAP kinase in response to inflammatory cytokines

or stress, leading to stress-induced cell cycle arrest and apoptosis. Expression of this kinase is

19

355 elevated in the white adipose tissue of obese individuals, and its inhibition may promote

356 adipose tissue browning and increase organismal energy expenditure (39). MVD encodes

357 mevalonate diphosphate decarboxylase, which regulates an early step in cholesterol

358 biosynthesis. MVD was also found to be upregulated in mice treated with an anti-obesity

359 therapeutic (40) or a Utx (Ubiquitously transcribed tetratricopeptide repeat, X chromosome, a

360 regulator of lipid metabolism)-demethylase knockout (41), with reduced triacylglycerol

361 synthesis but increased cholesterol synthesis. Of the genes that were significantly

362 downregulated in the "ongoing lipogenic state", notably the hepatic expression of HSD17B6

363 was also lowered in obese women (42).

364

365 In the "lipid storage state", quercetin and ferulic acid, in addition to attenuating lipid content,

366 changed the lipid composition, with more changes occurring when quercetin and ferulic acid

367 were combined. From the transcriptomics data, functions related to lipid metabolism such as

368 lipid handling, lipid accumulation and obesity development, were consistently regulated, with

369 strong predicted negative activation z-scores. NFATC2, associated with lipid synthesis, was

370 upregulated by ferulic acid and by the combined treatment, while ABCA1, a regulator of

371 intracellular cholesterol and HCER (43), was downregulated. Together with the lipidomics

372 data, this may contribute to the lower lipid accumulation (44-46). Pathway analysis showed

373 that the regulated transcripts are predicted to be controlled by the PPARs, the master

374 regulators of adipogenesis (47, 48), specifically nuclear PPARα and/or RXRα receptors, but

375 not PPARγ. For all three (poly)phenol treatments, the PPARα/RXRα-regulated genes

376 ABCA1, CD36 (cluster of differentiation 36/fatty acid translocase) and LPL (lipoprotein

377 lipase) genes were downregulated. The targeted deletion of ABCA1 in mouse adipocytes

378 decreased diet-induced obesity, lowering lipogenesis and triglyceride accumulation (44).

379 CD36 and LPL have roles in importing and promoting cellular uptake of free fatty acids (49)

380 and reduced expression of these three resultant proteins would be predicted to be associated

381 with reduced ability to accumulate and concentrate intracellular lipid. Overexpression of LPL

382 specifically in mouse skeletal muscle or liver has been shown to increase triacylglycerol

383 content in cells of theses tissues (50), whilst CD36 has been proposed to have a regulatory

384 role in fat gustatory perception in humans (51). Quercetin also regulated FASN (fatty acid

385 synthase), and (poly)phenol supplementation has been shown to significantly reduce body

386 mass and fat pad mass of rats fed a high fat diet with an associated significantly reduced

387 expression of FASN mRNA (52). Ferulic acid regulated PRKAR2B (protein kinase cAMP-

388 dependent type II regulatory protein β–subunit), and genetic disruption of PRKAR2B in mice

389 has been shown to be linked with limited ability to gain weight on a high fat diet and

390 increased energy expenditure of the modified mammals (53).

391

392 Whilst we show that treatment of SGBS cells in the "lipid storage state" by three low doses

393 of quercetin, ferulic acid or both significantly reduced the amount of neutral lipids, much

394 higher concentrations were required to induce this effect in the "ongoing lipogenesis state",

395 where lipogenesis is dominant. The PE (O-16:0/18:2) fraction was increased by ferulic acid,

396 and an increase in this species was similarly measured in the subcutaneous adipose tissue of

397 mice on a chow-fed diet versus those on a high-fat diet (54). Conversely, with quercetin and

398 ferulic acid, we also observed a shift in lipid composition towards more unsaturated TAGs

399 and non-storage lipids, seen in white adipose tissue of the mice following a high-fat diet (54),

400 and an increase in the ceramide fraction, which is commonly known to be associated with

401 metabolic syndrome risk (55). Nevertheless, our transcriptomics data suggest an overall

402 decrease in lipid synthesis, accumulation and conversion, gene expression changes consistent

403 with the super-pathway of cholesterol biosynthesis, rather than with PPARα/RXRα signalling

404 as in the "lipid storage state". Reduced cholesterol biosynthesis activity was associated with

405 quercetin, whether treated individually or combined, and AMP-activated protein kinase

406 (AMPK) signalling and reduced leptin expression was associated with ferulic acid. Overall,

407 these outcomes suggest a decrease in adipose expansion and lipid storage. Free fatty acid

408 fractions were decreased with all (poly)phenol treatments and this is associated with a lower

409 risk of insulin resistance, inflammation and metabolic syndrome (56).

410

411 In SGBS cells, a high concentration of quercetin (25 µM) for 16 h induced strong

412 downregulation of mRNA for PFKB, the gene for 6-phosphofructokinase isozyme 2, and the

413 PPAR-regulated ANGPTL4 gene product (angiopoietin-like 4) (14), a secreted protein

414 induced by glucocorticoids that inhibits LPL and stimulates intracellular adipocyte lipolysis

415 (57); taken together downregulation of these proteins may reduce the accumulation of free

416 fatty acids, and the associated lipotoxicity and inflammation, in insulin-resistant adipose

417 tissue. It has previously been reported that quercetin treatment during differentiation of pre-

418 adipocytes can reduce adipogenesis of murine 3T3-L1 murine cells (58). Relatively high *in*

419 *vivo* dosing regimens have been reported to promote anti-obesity effects in mice (3-5).

420 (Poly)phenols can also have other effects on adipose, where 3T3-L1 cells and rat adipose

421 tissue can be remodelled ('browned') to exhibit a more metabolically active brown-like

422 adipose cell phenotype (59, 60). Polyphenol treatment promoting adipocyte remodelling to a

423 brown cell-like phenotype has been shown to occur alongside changes in gene expression for

424 UCP1 (uncoupling protein 1), PPARγ coactivator 1α and FGF21 (fibroblast growth factor 21)

425 (59, 61). A consequence of browning may be increased triglyceride-derived fatty acid uptake

426 by white adipose (62).

427

428 In summary, (poly)phenols appear to more strongly enhance the burning of fat, but with

429 weaker effects on the synthesis of fat since in adipocytes that are actively making lipids, then

22

430   a much higher concentration is needed to lower the rate of synthesis. The effects on cells

431   storing lipid was through PPARα/RXRα activation, but these transcription factors were not

432   involved in cells actively undergoing lipogenesis.

Author Contributions

GW, AK and RL designed the study. RL performed experimental work, analysed data and drafted the manuscript. MJH performed 2-deoxy-D-glucose uptake experiments, contributed to experimental planning and interpretation of results, and helped draft the manuscript. IMC performed transcriptome sequencing. MW provided essential tools (SGBS cells). AK contributed to transcriptome analysis. GW and AK obtained funding. RL, MJH, AK and GW edited the manuscript. All authors approve submission.

TABLES

Table 1: **The effect of treatment on the mole fraction of lipid species in SGBS cells under "lipid storage state" conditions**

Molecular Species	Quercetin	Ferulic Acid	Quercetin + ferulic acid	Affected by Differentiation
MAG (17:0)	N.S	N.S	90 *	no
MAG (22:0)	24.6 **	N.S	35.4 *	yes
TAG 48:0-FA 14:0	70.7 *	N.S	N.S	no
TAG 44:0-FA 16:0	N.S	77.9 *	N.S	yes
TAG 44:0-FA 12:0	N.S	75.0 *	75.0 *	yes
TAG 47:1-FA 17:0	N.S	78.6 *	N.S	no
FFA (14:1)	N.S	163.6 *	N.S	yes
PC (16:0/12:0)	N.S	N.S	65.0 *	no
PC (16:0/16:0)	N.S	N.S	123.6 *	yes
PC (16:0/18:0)	N.S	N.S	95.6 *	no
PC (16:0/18:3)	N.S	N.S	67.9 *	no
PC (17:0/18:2)	N.S	N.S	63.9 *	no
PC (18:1/20:5)	N.S	N.S	170.8 **	no
PC (18:2/18:3)	67.1 *	N.S	N.S	no
PC (18:2/22:6)	75.0 *	N.S	N.S	no
PE (O-16:0/20:4)	N.S	N.S	123.5 **	no
PE (P-16:0/20:4)	N.S	N.S	123.8 *	no
PE (16:0/22:6)	131.3 *	N.S	125.0 *	no
PE (18:0/20:1)	N.S	N.S	83.3 *	no
LPC (16:0)	N.S	85.8 *	N.S	no

The effect of treatment (all 3×1 µM for 54 h) is expressed as a percentage of the vehicle control (100%). Mole fraction is the relative amount of a particular species as a percentage of all lipid species of that lipid class. All other detected metabolites (not listed) were not significantly affected by any treatment. n=5. N.S, not significant. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 2: **Total number of transcripts regulated significantly**

Treatment	Lipid storage state		Ongoing lipogenesis state	
	Expression increased	**Expression decreased**	**Expression increased**	**Expression decreased**
1 µM quercetin	216	84	140	87
1 µM ferulic acid	211	96	33	12
1 µM quercetin and ferulic acid	365	148	162	55

Expression is determined relative to detection of transcripts in vehicle-treated control cells

Table 3: **Genes with altered expression after treatment in the "lipid storage state" which are significantly associated with lipid handling pathways.**

Treatment	Change	Lipid synthesis	Lipid accumulation	Concentration of lipid
1 µM Quercetin	up	HMOX1	SPHK1, UCGC, HMOX1	SPHK1, UCGC, NGF, HMGA1, HMOX1
	down	SREBF1, THRSP, PNPL3A, GPAM, ACAC3, PRKD1, LPL1, LPIN1, IGF1, FASN, LTF	SREBF1, SPHK1, IGF1, FASN	SREBF1, THRSP, GPAM, SPHK1, LPL1, LPIN1, IGF1, FASN
1 µM Ferulic Acid	up	NFATC2, SIPR3, APOC1, CXCL8, SPHK1, UCGC, F2RL1, FOSL1, LIF	HMOX1, SPHK1, UCGC	HMOX1, SPHK1, UCGC
	down	CNTFR	ALDH1L1, DHRS3, FABP4, IGF1	ABCA1, IGF1, LPL, GPAM, THRSP
Combined treatment	up	SIPR3, UGCG, CCL2, CYP1B1, F2RL1, LIF, NFATC2	HMOX1, SPHK1, UGCG	HMOX1, SPHK1, UGCG, HMGA1
	down	APOC1, LTF	ALDH1L1, DHRS3, FABP4, IGF1	ABCA1, IGF1, LPL, ADORA1, GPAM, THRSP

n = 3 for each treatment.

Table 4: **The effect of treatment on the mole fraction of lipid species in the "ongoing lipogenesis state".**

Molecular Species	Quercetin	Ferulic Acid	Quercetin + ferulic acid	Affected by differentiation
DAG (12:0/16:0)	N.S	N.S	61.5 *	no
DAG (12:0/18:1)	N.S	N.S	78.6 *	no
DAG (14:0/16:0)	N.S	N.S	79.0 *	yes
DAG (14:0/18:2)	N.S	N.S	76.0 **	no
TAG 42:0-FA 16:0	73.5 *	N.S	73.5 *	yes
TAG 44:0-FA 12:0	N.S	N.S	75.8 *	yes
TAG 44:1-FA 18:0	80.0 *	N.S	N.S	no
TAG 46:0-FA 12:0	N.S	N.S	66.9 *	no
TAG 46:1-FA 12:0	N.S	N.S	80.0 *	no
TAG 50:5-FA 18:2	114.3 *	N.S	N.S	no
TAG 52:3-FA 16:0	120.0 *	N.S	N.S	no
TAG 52:3-FA 18:2	115.6 *	N.S	N.S	no
TAG 52:3-FA 20:2	127.7 **	N.S	N.S	no
TAG 52:3-FA 22:1	N.S	117.6 **	N.S	no
TAG 52:5-FA 18:2	N.S	141.7 *	N.S	no
FFA (14:1)	46.3 *	N.S	N.S	yes
FFA (15:0)	68.4 **	76.9 *	70.5 **	yes
CE (18:4)	154.0 *	162.0 *	N.S	no
PC (16:0/16:0)	127.6 *	N.S	124.6 *	yes
PC (18:0/14:1)	N.S	N.S	18.1 *	no
PE (O-16:0/18:2)	N.S	533.3 *	N.S	no
PE (18:1/22:4)	85.4 *	N.S	N.S	no
HCER (18:1)	N.S	129.4 *	N.S	no
DCER (24:1)	N.S	N.S	112.4 *	yes

The effect of treatment is expressed as a percentage of the vehicle treatment (100%). Mole fraction is the relative amount of a particular species as a percentage of all lipid species of that lipid class. All other detected metabolites (not listed) were not significantly affected in all 3 treatments. n = 5. N.S, not significant. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 5: **The effect of treatment on adipocytes in the "ongoing lipogenesis state" on gene expression related to lipid handling pathways.**

Treatment	Change	Lipid synthesis	Lipid accumulation	Conversion of lipid
1 µM quercetin	up	IGFBP4	SPHK1	SPHK1
	down	HMGCR, C3, FDPS, MID1PT	HMGCR, IGF1	HMGCR, IGF1, ADH4
Combined treatment	up	HMOX1, HMGA1	HMOX1	HMOX1
	down	HMGCR, IGF1, CD36	HMGCR, IGF1	HMGCR, ADH4, PTGER3

No changes relevant to lipid handling were seen in cells treated with 1 µM ferulic acid. n = 3 for each treatment.

FIGURE LEGENDS

Fig. 1: Experimental set up for promotion of differentiation and subsequent treatment of SGBS cells

Maturation of SGBS cells was promoted by changes of media every 4 d with cells receiving their first treatment on day 14 (T = 0 h). Basal medium was DMEM/F-12 medium supplemented with 1% (v/v) penicillin-streptomycin solution, 1% (v/v) pan-bio solution. Growth phase: Basal medium with 10% (v/v) fetal bovine serum. Adipogenesis phase: Basal medium supplemented with 125 nM transferrin, 20 nM human insulin, 100 nM cortisol, 0.2 nM triiodothyronine (T3), 25 nM dexamethasone, 250 µM 3-isobutyl-1-methylxanthine and 2 µM rosiglitazone. Lipogenesis phase: Basal medium supplemented with 125 nM transferrin, 20 nM insulin, 100 nM cortisol and 0.2 nM T3. Treatment phase: At 14 d, cells were treated under two conditions: "ongoing lipogenic state" with basal medium supplemented with 125 nM transferrin, 20 nM insulin, 100 nM cortisol and 0.2 nM T3, or "lipid storage state" with cells in basal medium. Treatments were vehicle (DMSO), quercetin, ferulic acid or a combined quercetin and ferulic acid treatment, at time zero (day = 14 d) and then every 24 h for up to 48 h (Time = 0, 24 and 48 h). At t = 54 h, cells were harvested for transcriptomic analysis, and after t = 72 h, for lipidomic analysis.

Fig. 2: Differentiated SGBS cells display a mature adipocyte phenotype

Differentiation of pre-adipocytes initially promotes morphological changes of SGBS cells (A). Storage of lipid is seen in cells differentiated for 2 weeks or longer (Bi & Bii) and is confirmed as lipid deposits by Oil Red O staining (Biii). Example images shown at each time point. Scale bar represents 200 µm, and all images are of same magnification. Differentiated SGBS cells (15 d) exhibit significant insulin-sensitive glucose uptake (C). n = 6 for each group. Lipid accumulation significantly increases between day 14 and 17 when

differentiation factors are maintained in culture ("ongoing lipogenesis state), but no

significant further lipid deposition is evidenced when differentiation factors are removed

from the culture media ("lipid storage state") (D). n = 9 biological replicates for each group.

Data expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$.

Fig. 3: Treatment does not significantly affect cell number or viability of differentiated SGBS cells.

The number of live SGBS cells (A) and cell viability using trypan blue exclusion (B) were

not significantly affected by (poly)phenol treatment (3×24 h treatment). Mean ± SEM, n =

5.

Fig. 4: Quality of transcriptome data for assessing treatment effects on gene expression

The 3 samples selected for analysis of transcript expression show limited batch variation.

Samples analysed from 54 h treated vehicle (A541-3) and 54 h 1 µM quercetin with 1 µM

ferulic acid (D541-3) in lipid storage conditions are presented (A). Detection of significantly

altered transcripts (in red) is independent of their level of expression and follows a normal

distribution. Transcripts from 54 h 1 µM quercetin with 1 µM ferulic acid relative to

transcript expression in 54 h vehicle-treated cells in lipid storage conditions are shown (B).

Principal component analysis (PCA) showed that one factor contributes 89% of the total

variance when assessing the effect of the combined 1 µM quercetin with 1 µM ferulic acid

treatment (D54, light blue) compared to vehicle treatment (A54, red) for cells treated for 54 h

in the "lipid storage state" (C).

Fig. 5: Changes in the mole fraction of lipid species in SGBS cells in the "ongoing lipogenic state" relative to the "lipid storage state"

Only changes are shown where the level of each individual lipid species was significantly different ($p < 0.05$) in the "ongoing lipogenic state" compared to the "lipid storage state"; ** $p < 0.01$; * = $p < 0.05$). The abundance of a lipid species is expressed relative to all lipids in that class and is expressed as species fold change (from 1.0) within the respective lipid class. No significant effect ($p > 0.05$) of ongoing lipogenesis was detected for changes in total lipid class, expressed as fold change of total lipids detected, shown in the column on the right-hand side. Magnitude of changes are proportional to colour intensity of the bars; increased fold change shown as red bar or highlight and decreased fold change as blue bar or highlight. n = 5 independent experiments.

Fig. 6: Effect of treatment on neutral intracellular lipid deposits in the "lipid storage state"

1 µM quercetin, 1 µM ferulic acid or a combined treatment, each for three sequential 24 h doses, significantly reduces lipid deposits from differentiated SGBS cells cultured in lipid storing conditions (A). n ≥ 9 biological replicates for each treatment. No significant effects of two 24 h (poly)phenol treatments (B) (n = 8 biological replicates for each group) or one 72 h treatment (C) (n = 9 biological replicates for each group) were found on stored lipid. V = vehicle, Q = quercetin, Fr A = ferulic acid. Data shown as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Fig. 7: Chronic (poly)phenol treatment in the "lipid storage state" drives changes in gene expression related to lipid handling via PPAR/RXR signalling

(A) The number of individual transcripts modified by 3 × 24 h (poly)phenol (1 µM of each) treatments. (B) Gene changes associated with (poly)phenol treatments are significantly (P<0.001, threshold line) related to lipid handling systems and metabolic conditions. For each

category, top bar = 1 µM quercetin, middle bar = 1 µM ferulic acid and lower bar = quercetin + ferulic acid (1 µM each).

Relationship predicted by IPA showing how quercetin (C), ferulic acid (D) and quercetin + ferulic acid (E) treatment (1 µM each) impact on lipid handling in the "lipid storage state". Red and green fill show increased and decreased gene expression respectively. The predicted effect of altered gene expression on lipid handling is shown by blue (inhibition), orange (activation), grey (undetermined) or yellow (inconsistent with obtained data) arrows. The predicted effects of canonical pathway signalling (CP), PPARα/RXRα, on the gene changes detected in chronic quercetin (F), ferulic acid (G) and combined (H) treated SGBS cells. Pink highlighted genes are strongly related to lipid handling. Other colour coding as for C-E. n = 3 biological replicates; **a** = lipid accumulation; **b** = lipid synthesis; **c** = lipid concentration.

Fig. 8: Neutral lipid accumulation is attenuated by combined (poly)phenol treatment at a higher concentration during the "ongoing lipogenic state".

Chronic treatment (3 × 24 h doses) with 1 µM (poly)phenol has no significant effect on lipid accumulation during ongoing lipogenesis (A.) n ≥ 9 biological replicates for each treatment. Combined 10 µM quercetin and 10 µM ferulic acid chronic treatment, but not individual 10 µM treatments, significantly attenuates lipid accumulation during the promotion of ongoing lipogenesis SGBS cells (B.) n = 9 biological replicates for each treatment. V = vehicle, Q = quercetin, Fr A = ferulic acid. Data shown as mean ± SEM. $**P < 0.01$.

Fig. 9: Chronic (poly)phenol treatment during the "ongoing lipogenic state"

The number of individual transcripts can be modified by one or multiple chronic (poly)phenol treatments (A). Gene changes associated with (poly)phenol treatments are significantly ($p < 0.001$, orange threshold line) related to lipid handling systems and

metabolic conditions (B). For each system, the top bar represents 1 µM quercetin, middle bar represents 1 µM ferulic acid and lower bar represents quercetin + ferulic acid treatment. Panel C and D show genes altered by quercetin and quercetin and ferulic acid treatment respectively are predicted to impact on lipid handling. Red and green fill show increased and decreased gene expression respectively. The predicted effect of altered gene expression on lipid handling is shown by blue (inhibition), orange (activation), grey (undetermined) or yellow (inconsistent with obtained data) arrows. n = 3 biological replicates; **a** = lipid accumulation; **b** = lipid synthesis; **c** = lipid concentration.

FIGURES

Fig. 1

A.

Day 0   Day 4   Day 8

Bi   Bii   Biii

Day 14   Day 17   Day 17

C. 2-DG uptake (µg 2-DG/mg protein) — vehicle, + insulin, **

D. lipid deposition (% of day 14 ammount) — Days differentiated: 14, 17, 14 + 3 basal, *

Fig. 2

A.

B.

Fig. 3

Fig. 4

fold change

Fig. 5

Fig. 6

A quercetin   ferulic acid

53   17   22

197

33   71

212

quercetin + ferulic acid

Fig. 7A

B

threshold -log (*P* value)

Categories (top to bottom): Lipid metabolism, Small molecule biochemistry, Molecular transport, Metabolic disease, Carbohydrate metabolism, Nutritional disease, Energy production, Free radical scavenging

Fig. 7B

42

Fig. 7C

D

Fig. 7D

Fig. 7E

Fig. 7F

Fig. 7G

H

Fig. 7H

A.

B.

Fig. 8

A quercetin ferulic acid

92   3   1

35

97   6

79

quercetin + ferulic acid

Fig. 9A

Fig. 9B

Fig. 9C

Fig. 9D

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