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Bioactive Components from Flowers of *Sambucus nigra* L. Increase Glucose Uptake in Primary Porcine Myotube Cultures and Reduce Fat Accumulation in *Caenorhabditis elegans*

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ABSTRACT: Obesity and insulin resistance in skeletal muscles are major features of type 2 diabetes. In the present study, we examined the potential of *Sambucus nigra* flower (elderflowers) extracts to stimulate glucose uptake (GU) in primary porcine myotubes and reduce fat accumulation (FAc) in *Caenorhabditis elegans*. Bioassay guided chromatographic fractionations of extracts and fractions resulted in the identification of naringenin and 5-*O*- caffeoylquinic acid exhibiting a significant increase in GU. In addition, phenolic compounds related to those found in elderflowers were also tested, and among these, kaempferol, ferulic acid, *p*-coumaric acid, and caffeic acid increased GU significantly. FAc was significantly reduced in *C. elegans*, when treated with elderflower extracts, their fractions and the metabolites naringenin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-5″-acetylglycoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, and isorhamnetin-3-*O*-glucoside and the related phenolic compounds kaempferol and ferulic acid. The study indicates that elderflower extracts contain bioactive compounds capable of modulating glucose and lipid metabolism, suitable for nutraceutical and pharmaceutical applications.

KEYWORDS: *elderflowers, elderflower extracts, type 2 diabetes, glucose uptake, fat accumulation, obesity, naringenin, kaempferol, phenolic compounds*

INTRODUCTION

Type 2 diabetes (T2D) and visceral obesity have been strongly implicated in the occurrence of a collection of interrelated metabolic abnormalities, commonly called the metabolic syndrome (MS).[1,2] T2D is commonly characterized by hyperglycaemia and hyperinsulinemia and is often accompanied by β-cell failure and enhanced gluconeogenesis in the liver.[3,4] Again, insulin sensitivity has been found to correlate negatively with fat accumulation (FAc), irrespective of age and genetic background.[5] Unfortunately, some of the available medications for T2D are associated with several undesirable side effects.[6] This has increased the need for the discovery of supplementary nutraceuticals with antidiabetic properties, capable of modulating both glucose and lipid metabolism.

Flowers of black elder are used in many European countries for their appealing flavor and native flower aroma, to make extracts, which are consumed as a beverage.[7,8] *Sambucus nigra* L. (black elder) concoctions have also been used as an alternative medicine against common cold and influenza.[9] Most of the studies on black elder have been performed on the fruits of the plant (elderberries) which is known for its antiviral and immunity-boosting effects,[10,11] but recent research has revealed that the flowers of black elder (elderflowers) have potential antidiabetic properties.[12] Elderflower extracts have been found to activate peroxisome proliferator-activated receptors and enhance insulin-dependent glucose uptake (GU) in adipocytes.[13] Moreover, in an observational study, elderflowers in combination with *Asparagus officinalis* have shown significant weight-reducing potential.[14]

Skeletal muscle is the primary site for glucose disposal, where about 75% of the insulin-driven glucose disposal takes place.[15] Therefore in this study we have examined the potential of elderflower extracts, fractions, and secondary metabolites to stimulate GU in primary myotube cultures. Our investigation resulted in the identification of potential bioactive flavonoids and phenolic acids from elderflower extracts and fractions. This motivated us to test additional phenolic compounds that are structurally related to the flavonoids and phenolic acids found in elderflowers and gather information on their potential antidiabetic effects. These additional phenolic compounds (kaempferol, ferulic acid, *p*-coumaric acid, and caffeic acid) are referred to in this article as "related phenolic compounds".

Stress can result in increased glucose utilization and uptake.[16] Again, oxidative stress has been implicated as a contributing factor in MS.[17] We therefore monitored the effect of

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elderflower extracts on (a) the transcription regulation of heme oxygenase 1 (HMOX1), a marker of oxidative stress response, and heat shock protein 70 (HSP70), whose expression has been found to be induced by different kinds of cellular stress conditions, including nutritional stress[18] and (b) generation of reactive oxygen species (ROS).

Together with a reduction in blood glucose levels, reduction of body fat is also an important goal for combating obesity and MS. In the worm *Caenorhabditis elegans* (*C. elegans*), most of the more than 400 genes involved in fat storage are evolutionarily conserved and act in common cellular pathways.[19] It has been used in several studies to show the effect of nutritional perturbations on obesity and other metabolic diseases.[20] The wild type strain, N2, was used in this study to examine the accumulation of the lipophilic dye Nile Red. While *C. elegans* is feeding on *Escherichia coli* bacteria, fluorescent compounds are primarily deposited in the fat storage compartments in the intestine.[21] A measure of the level of fat storage is obtained by comparing the fluorescence levels in untreated and treated worms. The present study demonstrates the effect of elderflower extracts and its selected secondary metabolites as well as some related phenolic compounds on GU in primary porcine myotubes and on FAc in *C. elegans*.

MATERIALS AND METHODS

Pigs used for the isolation of satellite cells were treated according to the Danish Ministry of Justice Law, no. 382 (June 10, 1987).

Preparation of Plant Extracts. Elderflowers (Caprifoliaceae, *Sambucus nigra* L. cv. Haschberg; Holunderhof Helle, Thumby, Germany) were picked in June 2007 and frozen immediately after harvest at −22 °C. The frozen flowers (5 kg) were homogenized and extracted using dichloromethane (DCM, 12 L) and subsequently methanol (MeOH, 10 L), overnight in the dark at 5 °C and filtered afterward. The extracts were dried under a vacuum, yielding 28.4 and 151.4 g of dry matter respectively. Liquid chromatography (LC) with photodiode array detector (PDA) and mass spectrometric detection was performed with LC-PDA-MS settings as previously described.[22]

Fractionation of Plant Extracts. A part of the DCM extract (11 g) was separated by flash CC (70 mm i.d., 400 g silica gel 63−200 μm Merck) using the following solvent gradient: 100% hexane (1 L), 10−100% ethyl acetate (EtOAc) in hexane in 10% steps (1 L each), 50:50 EtOAc-MeOH (1 L), yielding 112 fractions (100 mL each). The collected fractions were analyzed by normal phase thin layer chromatography (TLC) and then combined into seven fractions (DCM A−G). A part of the MeOH extract (5 g) was separated by flash CC (40 mm i.d., 100 g RP-18 silica gel) using the following solvent gradient: 100% H_2O (200 mL), 10% acetonitrile in H_2O (150 mL), 30−90% acetonitrile in H_2O in 20% steps (300 mL each), 100% acetonitrile (300 mL), yielding 39 fractions (50 mL each). The collected fractions were analyzed by reverse phase TLC and then combined into seven fractions (MeOH A−G).

Preparation of Samples for Bioassays. All extracts, chromatographic fractions, and standards were dissolved in dimethyl sulfoxide (DMSO 99.9%, Merck, Darmstadt, Germany) before they were tested for biological activity. Standards of naringenin, quercetin-3-*O*-rutinoside (Q-3-*O*-R), quercetin-3-*O*-glucoside (Q-3-*O*-G), quercetin-3-*O*-6″-acetylglucoside (Q-3-*O*-6″-A), kaempferol, kaempferol-3-*O*-rutinoside (K-3-*O*-R), isorhamnetin-3-*O*-glucoside (I-3-*O*-G), isorhamnetin-3-*O*-rutinoside (I-3-*O*-R), 5-*O*-caffeoylquinic acid (5-*O*-C), caffeic acid (CaA), *p*-coumaric acid (*p*-CA), and ferulic acid (FeA) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) or Extrasynthese (Genay, France).

Preparation of Myotube Cultures. Satellite cells were isolated from semimembranosus muscles of female pigs weighing approx. 12 kg, essentially as stated elsewhere[23] and stored in liquid nitrogen until used. To prepare myotube cultures, the cells were thawed and evenly seeded on Matrigel matrix (BD Biosciences, cat no. 354230) coated (1:50 v/v) 24, 48, or 96 well plates for RT-PCR studies, GU assay, and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) oxidation studies, respectively. Cells were proliferated in Porcine Growth Medium (PGM) consisting of 10% fetal calf serum (FCS), 10% horse serum, 80% Dulbecco's modified Eagles medium (DMEM) with 25 mM glucose (Life Technologies, Naperville, IL) and antibiotics (100 IU/mL penicillin, 100 IU/mL streptomycin sulfate, 3 μg/mL amphotericin B, 20 μg/mL gentamycin). The cells were grown in PGM until they were approximately 80% confluent in a CO_2-regulated humidified incubator (95% air and 5% CO_2 at 37 °C). Thereafter, the cells were proliferated to 100% confluence in media containing DMEM (7 mM glucose), 10% FCS, and antibiotics for 24 h and subsequently differentiated into myotubes by incubating with differentiation media (DMEM containing 7 mM glucose, 5% FCS, antibiotics, and 1 μM cytosine arabinoside) for at least 48 h.

Glucose Uptake Assay. The differentiated myotubes were treated with serum free media (DMEM with 7 mM, glucose, antibiotics, and 1 μM cytosine arabinoside) overnight, followed by incubation with various treatments and/or insulin (controls received equal volumes of DMSO) for 1 h. The myotubes were then washed with (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) HEPES buffered saline (20 mM Hepes, 140 mM NaCl, 5 mM KCl, 2.5 mM $MgSO_4$, 1 mM $CaCl_2$, adjusted to pH 7.4) and incubated with 0.1 mM 2-deoxy-[^{3}H] glucose (2 DOG) (250 μL/well) for 30 min; following which they were quickly washed with ice cold phosphate buffered saline (500 μL/well) and lysed by adding 0.05 M NaOH (37 °C, 250 μL/well) and placed on a shaking board for 30 min. The cell lysate was transferred to a scintillation tube, mixed with scintillation liquid (Ultima Gold, PerkinElmer Inc.) in 1:10 ratio and counted in a Win spectral, 1414 liquid scintillation counter (PerkinElmer, Life Sciences). The data was normalized with protein concentration per well.

mRNA Extraction and RT-PCR. Twenty-four well plates containing differentiated primary myotubes were exposed to treatment (200 μg/mL of elderflower DCM extract in differentiation media) for different time points in duplicates. After respective time intervals (1, 2, and 4 h), the myotubes were washed, harvested, and stored for later RNA extraction with RNeasy mini kit (Qiagen, Albertslund, Denmark). RT-PCR analysis was done with defined primers and probes described elsewhere.[24] The mRNA levels of HMOX1 and HSP70 were normalized against the reference gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). A standard curve was generated by serial dilution. Wells containing either water or genomic DNA were used as negative controls. All samples were measured in duplicate. The relative expression of HSP70 and HMOX1 were calculated according to the "Mathematical model for relative quantification in real-time PCR".[25]

Intracellular ROS. White-walled 96-well plates containing differentiated primary myotubes were treated, and the measured data were processed, essentially as has been mentioned elsewhere,[24] with the following changes. Background was measured before the addition of H_2DCF-DA. Myotubes were incubated with different concentrations of the elderflower DCM extract (0.1, 0.2, and 0.5 mg/mL) and H_2O_2 (100 μM in KCl buffer) separately. The intracellular oxidation of H_2DCF-DA in the wells was measured in quadruplicates at 37 °C for 4 h, at intervals of 4 min.

Fat Accumulation in *C. elegans*. The wild-type *C. elegans* strain N2 was used in this study. For fat staining experiments, Nile Red (N3013, Sigma-Aldrich, dissolved in acetone (500 mg/mL)) was added to molten nematode growth medium (NGM, ∼55 °C) to a final concentration of 0.05 mg/mL and aliquoted in 24-well plates (1 mL/well). The wells were seeded with *E. coli* bacteria (uracil auxotroph strain OP50) in 2× yeast extract tryptone medium (40 μL/well). When dry, 25 μL of M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 mL 1 M $MgSO_4$, H_2O to 1 L) and either 10 μL of DMSO (control) or 10 μL sample in DMSO were added. Synchronized L1 larvae were put on the plates and grown for 46 h at 20 °C until mid-L4 stage. Worms were then mounted in a drop of 10 mM tetramizole (T1512, Sigma-Aldrich) atop 2% agarose pads laid on a microscopy glass slide and overlaid with a coverslip. Fluorescence microscopy (rhodamine channel) was done using a Leica DMI6000 B microscope equipped

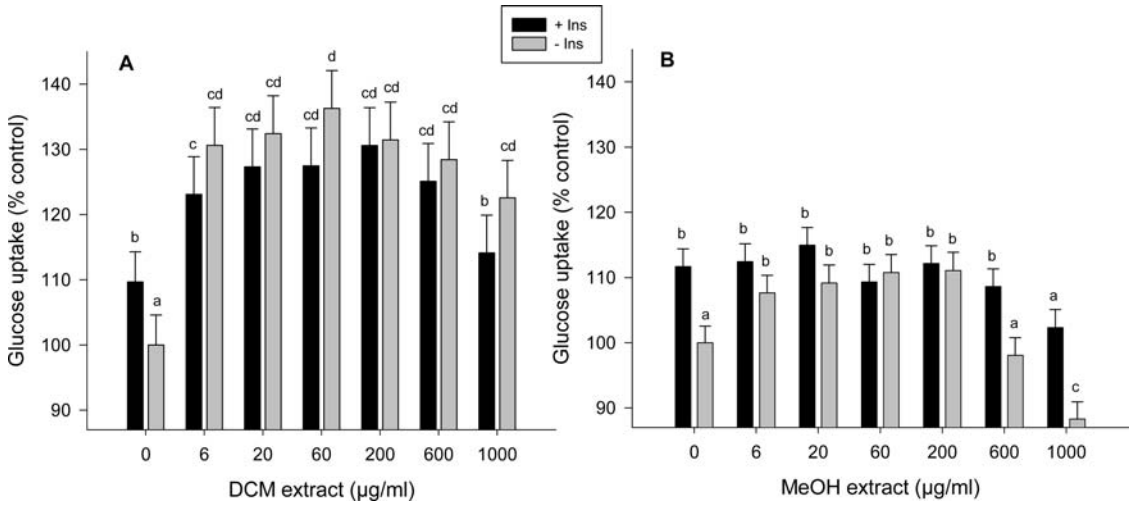

Figure 1. Effect of elderflower extracts on insulin-stimulated (750 pM) and insulin-independent glucose uptake in primary porcine myotube cultures exposed to (A) DCM extract and (B) MeOH extract. Glucose uptake is indicated as percent of the control (DMSO) which is set at 100. The plotted values are LSMeans ± SEM. *Y*-axis starts at 85 units. Ins = insulin. The letters (a, b, c, and d) on top of the bars indicate significant differences in glucose uptake. Number of pigs used = 3; number of replicates taken per pig = 6.

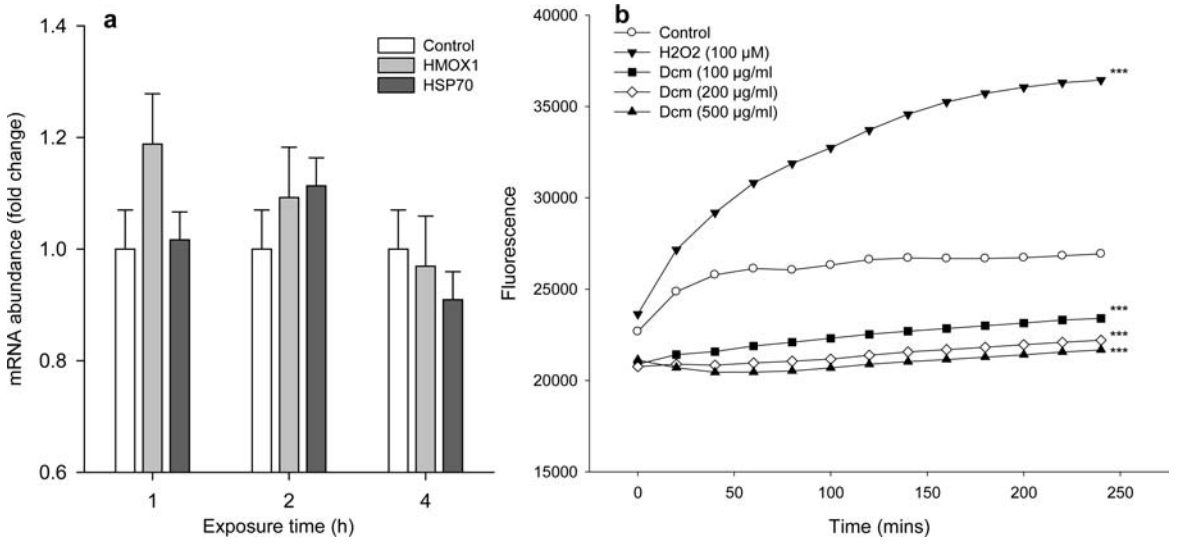

Figure 2. Elderflower extracts on stress responses in the primary porcine myotubes: (a) Effect of elderflower DCM extract (200 μg/mL) on the expression of heme oxygenase 1 (HMOX1) and heat shock protein 70 (HSP70) after 1, 2, and 4 h of exposure. The mRNA levels of HMOX1 and HSP70 have been normalized against the reference gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and are given in fold change relative to their respective controls at the given time points. *Y*-axis starts at 0.6 units. (b) Effect of elderflower DCM extract on generation of reactive oxygen species (ROS) determined by intracellular 2,7-dichlorofluorescein oxidation. The DCM extract were tested at 100, 200, and 500 μg/mL concentrations. H_2O_2 (100 μM) is used as a positive control. The *x*-axis shows every fifth data point. *Y*-axis starts at 15000 units. Plotted values are LSMeans ± SEM. Control = DMSO in media. Number of pigs used = 3; number of replicates taken per pig = 6. *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

with an Olympus DP71 camera. Images were captured using Visiopharm Integrator System software (Visiopharm, Denmark). All worms were photographed at 200× magnification and 30 ms exposure time. Images were quantified using ImageJ (http://rsbweb.nih.gov/ij/).

Data Analysis. For experiments on porcine myotubes, data were analyzed, using the "Mixed procedure" of SAS statistical programming software (Ver. 9.2; SAS Institute Inc., Cary, NC, USA). The model consisted of treatments and their interactions as fixed effects, and experiments, replicate determinations, and pigs within treatments as random effects. The level of HSP70 and HMOX1 mRNA expression were analyzed with a model where time points were considered as fixed effects and replicates as random effects. The number of pigs from which satellite cells were isolated varied between 3 and 4; the number of replicates was 6.

For studies on *C. elegans*, Student's unpaired *t*-test or Analysis of variance (ANOVA) was used. Statistical analysis was done with Graph Pad Prism 5 software package (GraphPad Software, Inc.) on the raw data before normalization of the data. Number of worms used for each treatment varied between 13 and 19.

RESULTS

The DCM and MeOH extracts of elderflowers were tested for their effect on both insulin-stimulated and insulin-independent GU in primary porcine myotube cultures (Figure 1). Both extracts were able to increase GU in muscle cells in the presence and absence of insulin. The DCM extract in particular

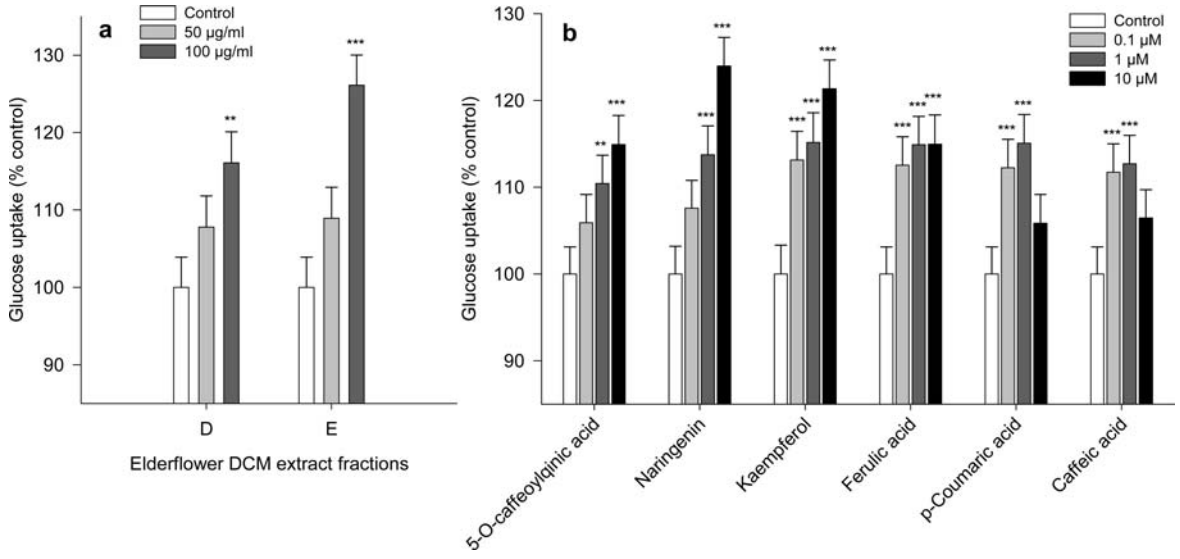

Figure 3. Effect of elderflower DCM fractions D and E, selected elderflower metabolites, and related phenolic compounds on glucose uptake in porcine myotubes. (a) Influence of the fractions D and E from elderflower DCM extract at the concentrations 50 and 100 μg/mL. (b) Effect of selected elderflower metabolites and related phenolic compounds tested at the concentrations of 0.1, 1.0, and 10 μM. Glucose uptake is indicated as percent of control (DMSO) set at 100. The plotted values are LSMeans ± SEM. *Y*-axis starts at 85 units. Number of pigs used = 3; number of replicates taken per pig = 6. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs control.

caused a significant increase in GU with respect to the control at all concentrations tested, the only exception being at 1000 μg/mL where the increase was not statistically significant (Figure 1a). The MeOH extract showed a small but significant increase in GU (other than at 600 and 1000 μg/mL) in the absence of insulin (Figure 1b). For the DCM extract, the increase in GU without insulin was statistically consistent within the tested concentration range of 6−1000 μg/mL, with a numerical maximum increase of 36% ($p < 0.0001$) at 60 μg/mL compared to control. In the presence of insulin, an increase of 21% ($p = 0.0006$) at 200 μg/mL was observed for the DCM extract. At the highest tested concentration (1000 μg/mL) of the DCM extract, the increase without insulin was 23% ($p < 0.0001$), and there was no significant increase in the presence of insulin. The effect of the elderflower MeOH extract was less prominent than that of the DCM extract. It did not show a significant increase in GU in presence of 750 pM Insulin. However, a significant increase of 7% ($p = 0.04$) to 11% ($p = 0.0003$) in GU, compared to the control at 6−200 μg/mL was observed in the absence of insulin. MeOH extract showed a significant decrease in GU at the maximum concentration used (1000 μg/mL), both in the presence and absence of insulin. The DCM extract showed much higher response in GU compared to MeOH, and was therefore chosen for further studies on stress responses. When tested for any detrimental effect on prolonged exposure, neither DCM nor MeOH extract showed any reduction in myotube viability (data not shown).

To examine whether the observed effect of the extracts on GU was caused through the induction of stress on the myotubes, mRNA abundance of HSP70 and HMOX1 was studied (Figure 2a). Exposure times of 1, 2, and 4 h and a concentration of 200 μg/mL were chosen in accordance with the exposure time for the GU assay. No significant up or down regulation of either HSP70 or HMOX1 mRNA was observed. Additionally, the influence of the DCM extract on the generation of intracellular ROS was determined at 100, 200, and 500 μg/mL (Figure 2b). A significant reduction in the formation of ROS was observed with respect to control at all the concentrations tested.

To aid identification of the bioactive components in the two extracts, they were separated by flash CC. Separation of each of the two extracts resulted in seven fractions (A−G). All 14 fractions obtained were then screened for their potential to induce GU independently at concentrations of 50 and 100 μg/mL. Two DCM fractions D and E were able to increase GU significantly at 100 μg/mL, by 17 and 26%, respectively (Figure 3a). None of the MeOH fractions showed any effect on GU. The DCM fractions D and E showed no reduction in myotube viability (data not shown).

The elderflower DCM and MeOH extracts obtained were characterized by LC-PDA-MS. Major components in the MeOH extract corresponded to those previously reported,[22,26] and the most prominent ones were 5-*O*-C, Q-3-*O*-R, K-3-*O*-R, and I-3-*O*-R. Major components of the DCM extract were α-linolenic acid (α-LA), linoleic acid (LA), naringenin, and some unknown phenolic acid derivatives. The DCM fractions D and E exhibited both an increase in GU and a decrease in FAc and were analyzed by LC-PDA-MS. Fraction D contained some unknown phenolic acid derivatives, α-LA, LA and epoxy-linalool; fraction E contained naringenin and α-LA as the major components, together with minor amounts of naringenin derivatives.

Since the bioactive fractions D and E of the DCM extract mainly contained naringenin, α-LA, and LA, these three compounds were selected for further studies together with several other known elderflower metabolites[22] and related phenolic compounds to further explore the observed bioactivities of the extracts and the fractions. Overall, the elderflower metabolites tested were naringenin, 5-*O*-CA, Q-3-*O*-R, Q-3-*O*-G, Q-3-*O*-6″-A, K-3-*O*-R, I-3-*O*-G, I-3-*O*-R, α-LA, and LA, and the related phenolic compounds tested were kaempferol, CaA, FeA, and *p*-CA. All were tested for effects on insulin-independent GU in primary porcine myotube cultures at the concentrations 0.1, 1.0, and 10 μM; other than α-LA and LA, which were tested at 10, 30, 70, and 100 μM. The

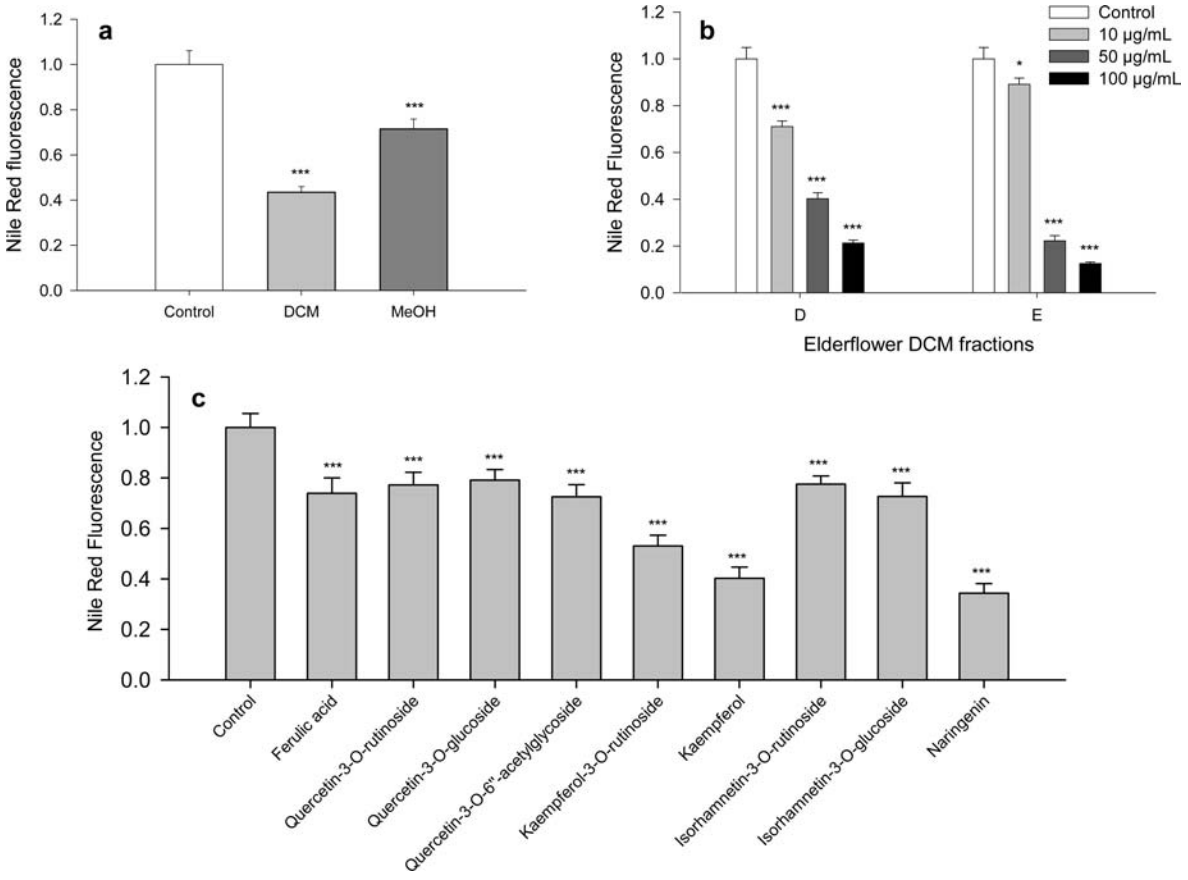

Figure 4. Effect of elderflower extracts, fractions, selected elderflower metabolites, and related phenolic compounds on *C. elegans* fat accumulation. Wild type worms from larval stage L1 to L4 were treated with extracts, fractions, or pure compounds on standard NGM plates containing the lipophilic dye Nile Red. Control worms only received DMSO. (a) Effect of DCM and MeOH extracts tested at the concentrations of 200 μg/mL. Number of treated worms = 13−19. (b) Effect of elderflower DCM extract fractions D and E. Worms were treated with 10, 50, and 100 μg/mL of plant material from D and E fractions. Number of treated worms = 5−10. (c) Effect of elderflower metabolites and related phenolic compounds. Worms were treated with the compounds at the concentration of 50 μM. Number of treated worms = 10. Fluorescence levels are shown as normalized means ± normalized SEM *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

compounds exhibiting a significant increase in GU are illustrated in Figure 3b. Among them, 5-*O*-CA was identified in the MeOH fractions; naringenin in the DCM fractions and kaempferol, FeA, *p*-CA, and CaA belong to the related phenolic compounds tested. None of the other compounds showed any effect on GU, although LA (in DCM fraction) showed a strong tendency (p = 0.056) at the concentration of 10 μM. The highest increase was observed at the concentration of 10 μM for naringenin and kaempferol (24% and 21%, respectively).

The effect of elderflower extracts, fractions, individual elderflower metabolites as well as related phenolic compounds on *C. elegans* fat storage is illustrated in Figures 4 and 5. Both MeOH and DCM extracts (200 μg/mL) decreased the Nile Red fluorescence (NRF) significantly by 25% and 50%, respectively, relative to control (Figures 4a and 5a). The DCM and MeOH fractions (A−G) were tested further. The MeOH fractions C−G (100 μg/mL) showed significant reduction in NRF (23−55%), with the maximum reduction by fraction F (55%, p < 0.0001) (data not shown). Among the DCM fractions tested at three different concentrations (10, 50, and 100 μg/mL), B, C, F, and G exhibited significant reduction in NRF (13 to 48%) (data not shown). But the highest reduction in NRF was observed for fractions D (78%) and E (87%) at a concentration of 100 μg/mL (Figures 4b and 5b). All the elderflower metabolites (other than α-LA, and LA) and related phenolic compounds mentioned above were tested for reduction in FAc at the concentration of 50 μM. The compounds exhibiting a significant decrease in NRF are illustrated in Figure 4c. Among them, Q-3-*O*-R, Q-3-*O*-G, Q-3-*O*-6″-A, K-3-*O*-R, I-3-*O*-G, and I-3-*O*-R were identified in the MeOH fractions, naringenin in the DCM fractions, and FeA and kaempferol belongs to the related phenolic compounds tested. None of the other compounds showed any effect on FAc. The highest reduction was observed for naringenin and kaempferol (65 and 60% respectively) compared to control (Figures 4c and 5c).

■ DISCUSSION

Insulin resistance, manifested by inadequate GU in major glucose utilizing tissues, and visceral obesity are two key aspects of T2D and MS. Elderflower extracts have previously been shown to induce weight loss and exhibit antidiabetic properties. This study showed for the first time that elderflower extracts can autonomously stimulate GU in primary myotube cultures and reduce FAc in vivo.

The elderflower DCM extract produced a much higher increase in GU in porcine myotubes, compared to the MeOH extract, and only the chromatographic fractions of this extract showed bioactivity in both GU and FAc studies in porcine myotube cultures and *C. elegans* respectively. Although the

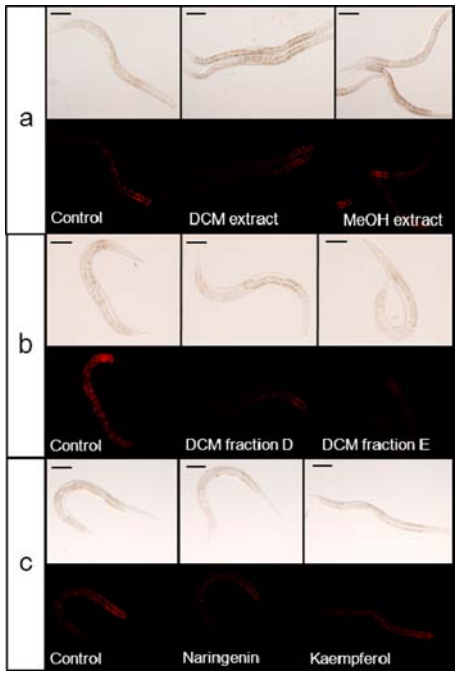

Figure 5. Effect of elderflower extracts, fractions, and selected phenolic compounds on staining of lipid stores in *C. elegans*. (a) Control (DMSO) and 20 μg/mL raw extracts of elderflower (b) control (DMSO) and 100 μg/mL fractions D and E of the DCM extraction of elderflower (c) control (DMSO) and 50 mM of pure standards naringenin and kaempferol. Upper panel in each row show differential interference contrast microscopy images; lower panel is the corresponding fluorescence images (rhodamine filter). Scale bar corresponds to 0.1 mm.

MeOH extract showed bioactivity, none of its fractions caused any increase in glucose uptake. Nevertheless, in FAc studies with *C. elegans*, several of the MeOH fractions were found to be potent. It is noteworthy that although the fractions are normally expected to contain larger concentrations of individual secondary metabolites compared to the extracts, the increase in GU induced by the DCM extract was higher compared to its bioactive fractions D and E. The reduction (in case of DCM extract) and loss (in case of MeOH extract) of bioactivity when fractionated indicates that part of the increase in GU exhibited by the extracts could be due to additive or synergistic effects, together with possible nonligand activation by some of the compounds present in the DCM and MeOH extracts, or antagonism by some other compounds present in relative higher concentrations in specific fractions, compared to the extract. Such observations are in agreement with synergistic interactions observed in separate studies carried out previously with both elderflowers and other plant extracts.[13,27] However, such reduction of activity was not observed for FAc studies, indicating a different mode of action and specificity of the elderflower metabolites.

It has been documented from earlier studies that cellular stress can result in an increase in glucose uptake.[16] However, gene expression analysis of HSP70 and HMOX1 genes and determination of intracellular ROS showed no indication of cellular stress on the myotubes. On the contrary, the DCM extract exhibited significant antioxidative properties by reducing the amount of intracellular ROS.

It has been observed throughout the tested concentration range of the DCM extract that, although it exhibits an increase in GU in the presence of a biologically relevant concentration of insulin (750 pM), it shows a higher increase in GU when administered in the absence of insulin. This indicates the possibility that the signaling molecules responsible for the observed increase in GU by the elderflower metabolites might be shared to some extent with the insulin signaling pathway.

Several fractions of the MeOH and DCM extracts showed a reduction in FAc in *C. elegans*. Unlike GU, further decrease in FAc was observed upon fractionation. Although many of the elderflower metabolites present in the MeOH extract showed significant reduction in FAc, naringenin (identified in the DCM extract) caused the highest reduction (Figure 4c).

The two fractions D and E of the DCM extract both exhibited an increase in GU and a decrease in FAc. Among the compounds present in these fractions were unknown phenolic acids, and further research is required to identify and elucidate their structures. However, among the compounds that were identified in these fractions, the most prominent increase in GU and decrease in FAc were observed for naringenin. Both confirming and contradictory findings exist, where naringenin was found to enhance GU in L6 myotubes via adenosine monophosphate-activated protein kinase (AMPK) activation[28] but reduce GU in MCF-7 breast cancer cells[29] and U937 cells.[30] The reported inhibitory effects of naringenin on GU can be explained by the different cell types used in these studies, implying a cell-type specific effect of this compound.

Among the related phenolic compounds tested, kaempferol showed the highest increase in GU and reduction in FAc. This is the first account demonstrating the ability of kaempferol to enhance GU in myotubes and reduce FAc in vivo. However, in separate studies, kaempferol has previously been shown to inhibit GU in HeLa cells[31] but enhance GU in mature 3T3-L1 adipocytes,[32] which is similar to the cell-type dependent response, observed for naringenin. It is important to note that although naringenin is a flavanone and kaempferol a flavonol, they are very similar in their chemical structures, that is, in size and the substitution pattern of their aromatic rings. This could signify the structural importance of these molecules in their observed regulation of glucose and lipid homeostasis.

In summary, it can be concluded that extracts, fractions, and several secondary metabolites from elderflowers possess pronounced bioactivities and can be used to modulate glucose and lipid metabolism. Studies conducted with the extracts, fractions, and their constituent compounds revealed that among the elderflower metabolites, naringenin is one of the most potent, with a major effect on the enhancement of GU and reduction in FAc. MS is characterized by elevated blood glucose levels and increase in visceral obesity, and the fact that elderflower extracts exhibit the potential to amend both of these metabolic defects qualifies it for further investigation for its application as a supplementary nutraceutical.

AUTHOR INFORMATION

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Notes

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■ ABBREVIATIONS USED

CaA, caffeic acid; FeA, ferulic acid; I-3-O-G, isorhamnetin-3-*O*-glucoside; I-3-*O*-R, isorhamnetin-3-*O*-rutinoside; K-3-*O*-R, kaempferol-3-*O*-rutinoside; 5-*O*-C, 5-*O*-caffeoylquinic acid; *p*-CA, *p*-coumaric acid; Q-3-*O*-G, quercetin-3-*O*-glucoside; Q-3-*O*-6″-A, quercetin-3-*O*-6″-acetylglucoside; Q-3-*O*-R, quercetin-3-*O*-rutinoside; ROS, reactive oxygen species; TLC, thin layer chromatography; NRF, nile red fluorescence; DCM, dichloromethane; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; H_2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; MeOH, methanol; MS, metabolic syndrome; FAc, fat accumulation; GU, glucose uptake

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