A trimer plus a dimer-gallate reproduce the bioactivity described for an extract of grape seed procyanidins

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ABSTRACT

The relationship between grape seed-derived procyanidin extract components and their bioactivity was explored. The monomeric and dimeric structures only acted as anti-inflammatory agents. Similarly, pure C1 trimer was highly effective on LPS-activated macrophages. To reproduce all of the bioactivities of the total extract, a fraction enriched with trimeric structures was needed. This trimeric-enriched fraction was divided into subfractions, the most bioactive of which contained two compounds with a molecular weight

equal to a trimer (865) and a dimer-gallate (729), according to spectrometric analysis. Thus, it may be concluded that a mixture of both molecules reproduces the bioactivity in glucose metabolism (3T3-L1), lipid metabolism (HepG2) and macrophage functionality (RAW 264.6).

Keywords: Dimer-gallate, Procyanidin, Trimer, Glucose uptake, Triacylglycerol secretion, Cholesterol secretion, Anti-inflammatory effect.

1. Introduction

The "French paradox" has attracted the attention of researchers worldwide for more than a decade (Renaud & De Lorgeril, 1992). Many studies have proved several positive and healthy effects of grape seed-derived procyanidins (GSPE) (Aron & Kennedy, 2008; Rasmussen, Frederiksen, Struntze Krogholm, & Poulsen, 2005). Most of them, however, have examined phenolic extracts derived from grape seed, because they are waste products of the winery and grape juice industry—a rich source of polyphenols—and because it is difficult to find individual compounds as pure structures in these extracts.

The composition of GSPE is known to consist largely of gallic acid, catechin, epicatechin and procyanidin dimers and trimers composed of flavan-3-ol units with C4-C8 or C4-C6 interflavan linkages (Agarwal et al., 2007). These compounds are also present as esters linked to gallic acid in the aliphatic 3-hydroxyl group in the C ring. Some attempts have been made to evaluate how effective the different components of these extracts are at improving some of the well-described properties of the whole extract, mainly growth inhibition and apoptotic death (Agarwal et al., 2007; Faria, Calhau, deFreitas, & Mateus, 2006; Lizarraga et al., 2007). Guo et al. (2007) proved that oligomeric and polymeric grape seed procyanidins are effective at protecting and treating ailments in the central nervous system induced by alcohol abuse.

Grape seed-derived procyanidin extracts have several healthful properties: for example, they act as antioxidants (Puiggròs et al., 2005), they improve lipid metabolism (del Bas et al., 2005), they limit

adipogenesis (Pinent et al., 2005), and they function as insulinomimetic agents (Pinent et al., 2004) and anti-inflammatory agents (Terra et al., 2007). This study aims to identify the structure(s) responsible for these healthful effects. This was achieved by two fractionation steps of the initial extract according to its effectiveness at improving several functions in three different cell lines. Once a highly effective fraction had been identified, its structures were characterised using HPLC-ESI-MS and the molecular weights of individual peaks were confirmed using MALDI-TOF.

2. Materials and methods

2.1. Cells, reagents and materials

The procyanidin extract contained 76% procyanidin with the following composition: 1.63% phenolic acids (mainly gallic acid), 20.92% monomers (mainly catechin + epicatechin), 20.71% dimers + epigallocatechin-gallate (EGCG), 17.33% trimers and 39.41% oligomeric forms of four units or more. Pure molecules were mostly obtained from SIGMA (Madrid, Spain). These were hippuric acid, ferulic acid, 3-hydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, p-hydroxyphenylacetic acid, vanillic acid, 3-hydroxyphenylacetic acid, p-coumaric acid, epigallocatechin, catechin gallate, epicatechin, gallocatechin gallate, epicatechin gallate, catechin hydrate and epigallocatechin gallate. Procyanidin B1–B4 came from APIN Chemicals (Abingdon, Oxon, UK). All procyanidin extracts, fractions, subfractions and pure molecules were prepared in absolute ethanol. Appropriate dilutions were made in order to obtain a 0.1% (v/v) ethanol concentration in all control and treated wells.

Cell culture reagents were obtained from BioWhittaker (Verviers, Belgium). Insulin (Actrapid) was from Novo Nordisk Pharma SA (Madrid, Spain). Bradford protein reagent was from Bio-Rad Laboratories (Life Science Group, Hercules, CA, USA), 2-deoxy-[1-3H]-glucose and 14C-acetate was from Amersham Biosciences (Buckinghamshire, England).

2.2. Chromatographic separation of procyanidin extract

GSPE (0.5 g) was subjected to normal-phase chromatography column (35–70 mesh, Interchim, Montluçon, France) preconditioned with solvent A (acetone/hexane, 65:35) as follows: GSPE components were separated according to size using an increasing gradient of solvent B (acetone/hexane, 80:20). First, low molecular weight compounds were eluted with solvent A, then the proportion of solvent B was gradually increased until it reached 100% after 1 h. Finally, an additional volume of solvent B was added, and 10 mL fractions were collected using a fraction collector. The fractions collected were monitored using TLC on PolyGram silica gel 0.2 mm with fluorescent indicator UV254 (Macherey-Nagel, Hoerdt, France), with the mixture toluene/acetone/acetic acid (3:3:1, v/v/v). The TLC plates were visualised following spraying with anisaldehyde reagent. Eleven major fractions with increasing degrees of polymerisation were identified, according to their retention time, Rf (Terra et al., 2007). These fractions were vacuum-dried and kept at -20 °C for subsequent use in the biological studies.

The most effective fraction (VIII) was further subfractionated by semipreparative HPLC (Varian, Model 210 Walnut Creek, CA, USA) with a 4 x 250 mm Ultrasep RP18 column (4 µm) (Bischoff, Leonberg, Germany) at room temperature using the following solvents: water/formic acid (95.5:4.5, v/v) (A) and acetonitrile/solvent A (10:90, v/v) (B) with the following gradient system: 0–40% B (0–10 min), 40–60% B (10–35 min), 60–100% B (35–50 min) and 100% B (50–60 min). Detection was carried out at 286 and 306 nm, with a UV–vis detector (Varian, Model 345, Walnut Creek, CA, USA).

An initial approach for determining molecular weight was to use HPLC-ESI-MS. A Platform II (Micromass, Manchester, UK) with electrospray injection (ESI) was used, coupled to the LC apparatus (reversed phase LC on a Waters TM system 600 E, Saint-Quentin, France). Procyanidins can easily shed a proton, generating intense negative ions [M-H]-, so detection was performed in the negative ion mode. A low voltage was used to avoid fragmentation; the products were identified by their molecular peaks.

The chromatogram peaks isolated by semipreparative HPLC were also characterised by MALDI-TOF. MALDI-MS spectra were obtained using a matrix-assisted laser-desorption ionisation-time-of-flight mass

92 spectrometer (TofSpec MALDI-TOF) from Micromass (Manchester, UK). This instrument has a pulsed
93 nitrogen laser (337 nm, 4 ns pulse width) and a time-delayed extracted ion source. Spectra were recorded
94 in the positive-ion mode using the reflectron and a 20 kV accelerating voltage.

95 **2.3. Cell culture and measurements**

96 3T3-L1 preadipocytes were cultured and induced to differentiate as previously described (Ardévol, Bladé,
97 Salvadó, & Arola, 2000). Ten days after differentiation, fully differentiated adipocytes were washed twice
98 with phosphate buffered saline (PBS) and incubated at 37 °C with serum-free supplemented Dulbecco's
99 modified Eagle medium (DMEM) containing 0.2% bovine serum albumin (BSA) (depletion medium) for
100 2 h. During the last 30 min of this depletion treatment, the cells were treated with GSPE or insulin.
101 Afterwards, glucose transport was determined by measuring the uptake of 2-deoxy-D-[3H] glucose, as
102 previously described by Pinent et al. (2004). Each condition was run in triplicate.

103 HepG2 cells (ATCC code HB-8065, Manassas, VA, USA) were propagated in DMEM and cultured as
104 previously described (Puiggròs et al., 2005). The only modification was the addition of 25 mM 4-(2-
105 hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (SIGMA, Madrid, Spain) to the culture media.
106 For the experiments, HepG2 cells were seeded at 750,000 cell/well in 12-well-plates and left to grow for
107 two days (80% confluence) in a propagation medium. The medium was replaced 16 h before treatment.
108 Then, lipid synthesis was measured. Procyanidins and 14C acetate (0.6 µCi/mL) were added to the cell
109 media and 6 h after treatment the media and cells were collected and the lipids were extracted using 3
110 volumes and 0.5 ml of hexane/isopropanol (3:2, v/v) respectively. Thin layer chromatography was
111 performed with petroleum either: diethyl ether: NH3 (40:10:0.1) and an additional separation using a
112 hexane/methyl tert-butyl ether (MTBE)/NH4OH (30:20:0.1, v/v/v) solvent to obtain the free cholesterol,
113 cholesterol esther and triacylglycerol (TAG) fractions. Each fraction was scraped and determined by
114 scintillation counting. Values were corrected per milligram of protein, determined using the Bradford
115 methodology (Bradford, 1976). The medium was collected after 24 h treatment and apolipoprotein B was

detected as described in del Bas et al. (2008).

Murine macrophage cell line RAW 264.7 (European Tissue Culture Collection, ECACC, Ref. 91062702, London, UK) was cultured as previously described (Terra et al., 2007) and used for experiments between passages 5 and 14. At 80% of confluence, adherent monocyte-RAW 264.7 cells were incubated with different compounds and with 1 μg/mL LPS simultaneously for 19 h. The culture medium for control and treated cells was collected and tested for nitric oxide (NO) and prostaglandin E2 (PGE2) production. The nitrite concentration in the culture medium was measured as an indicator of NO production according to the Griess reaction (Terra et al., 2007). The level of PGE2 released into culture medium was quantified and normalised as previously described (Terra et al., 2007).

2.4. Calculations and statistical analysis

Results are expressed as the mean ± SEM. Effects were assessed using Student's t-test. All calculations were performed using SPSS software.

3. Results and discussion

3.1. Monomeric and dimeric components of grape seed-derived extract act mainly as anti-inflammatory agents

The main objective of this work was to identify the molecule(s) responsible for the bioactivity of a grape seed procyanidin extract that act in vivo as (a) an antihyperglycemic (Pinent et al., 2004), (b) an antiatherogenic (del Bas et al., 2005, 2008) and (c) an anti-inflammatory (Terra et al., 2008) product. To meet this objective, three cell lines in which GSPE has been identified as being highly active, namely adipocytes (Pinent et al., 2005; Pinent, Bladé, Salvadó, Arola, & Ardévol, 2005a, 2005b), hepatocytes (del Bas et al., 2005; Puiggròs et al., 2005), and macrophage cells (Terra et al., 2007) were used. The effects that pure compounds have on these cells and the two fractionation steps have on the whole extract were evaluated.

The monomeric components of the extract have clearly been proven to reach body fluids and some of them have been modified in the body (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Thus, we hypothesise that these molecules or their modified forms are the main cause of the effects described. However, as Table 1 summarises, none of these pure monomeric compounds stimulated glucose uptake in the adipocytes, and HepG2 hepatocytes had no effect on TAG or total cholesterol secreted to the cell culture media while 25 mg/L GSPE inhibited TAG and TC secretion 45% and 25% respectively (results not shown). Neither did pure dimeric compounds act on the functions tested (Table 2). Both results agree with those found in the first fractionation step (Terra et al., 2007). Table 3 indicates that the fractions enriched with monomeric and dimeric compounds did not seem to affect adipocytes or hepatocytes. Monomeric components were previously reported not to induce lipolysis in adipocytes (Ardévol et al., 2000) or to protect against oxidative stress in Fao hepatocytes (Roig, Cascón, Arola, Bladé, & Salvadó, 2002). In both situations the total extract was highly effective. Only fractions III and V showed a slight but significant decrease in the amount of ApoB secreted to the cell culture media of HepG2 cells. Both fractions, together with fraction IV, share a peak at the end of the chromatogram that could be epicatechin gallate. In this respect, Yee et al. (2002) also found that, unlike EC, EGCG is a potent inhibitor of ApoB secretion, suggesting that the gallate moiety has a beneficial effect on the catechin molecule and that this is beneficial for lipid metabolism in terms of ApoB secretion.

Also, a common trait in the different approaches was that almost all monomeric (Table 1) and dimeric (Table 2) structures showed anti-inflammatory properties equal to the total grape seed procyanidin extract (Fig. 1) and in some cases had a stronger effect (i.e., EGCG limited NO production). These results agree with previously published works showing the anti-inflammatory effect of procyanidin B2 (Chen, Cai, Kwik-Uribe, Zeng, & Zhu, 2006; Zhang et al., 2006).

3.2. Oligomeric fractions of the extract justify its complete bioactivity

Park, Rimbach, Saliou, Valacchi, and Packer (2000) have shown that trimeric procyanidin C2 do not act

as an anti-inflammatory compound. The trimer C1 has now been examined and it has been shown not only to have a considerable anti-inflammatory effect (Fig. 1) but also to be active on hepatocytes and adipocytes (Table 2). C1 reproduces most of the bioactive effects of the total extract. We evaluated it as a pure compound at the same concentration of the total extract and found a much lower effect (GSPE inhibited 45% TAG secretion in HepG2). However, this is the first time a C1 procyanidin has been described as having a bioactivity that is different from its antioxidant activity, which was previously shown to be higher than the antioxidant activity of other smaller oligomeric structures (da Silva Porto, Laranjinha, & de Freitas, 2003).

Examination of the trimeric-enriched fractions in the extract examined here showed that fractions VIII–XIII positively activate all the biological functions (see Table 3). Fraction VIII almost completely lacks monomers and dimers, but has the greatest bioactivity in the extract tested. Another fractionation step was carried out on fraction VIII to reach the objective. Fig. 2a shows a chromatogram of those subfractions whose bioactivity was closer to the total of fraction VIII (S2, S3, S4 and S12) for both cell lines. Fig. 2b–d summarises all the results obtained with these subfractions. Several bioactive subfractions (S2, S3, S4, S6, S12 and S13) can be found effective for both cell lines. However, it should be taken into account that the bioactivity of each one of these subfractions was evaluated at the same concentration as the total initial extract in each cell line. This facilitates the comparison of the effects between subfractions, but also distorts the truth because the amount of each subfraction obtained was in the order of S13 (3.6 mg) > S12 > S11 > S10 > S7 > S6 > S8 > S4 > S5-S9 > S2 > S3 (0.5 mg). S12 was the biggest subfraction and it had the greatest effect, so it was selected for further analysis. Two complementary analyses were carried out: the fraction VIII was analysed with HPLC-ESI-MS and the isolated S12 subfraction with MALDI-TOF analysis. The results showed that S12 has two molecular weight components: a dimer-gallate (729) and a trimer (865), the two peaks found in the HPLC chromatogram (Fig. 2a). The trimer component has been assigned to C1 procyanidin by comparing retention times and because it is the most abundant form in grape seed (de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000). However, the dimer-gallate is

188 probably not the B2-gallate, which was identified at S13, a fraction that did not share a single peak with
189 S12 and which was barely active on the three assayed functions. Similarly, Schäfer and Högger (2007)
190 have demonstrated that the inhibitory action of Pycnogenol on a-glucosidase, in vitro, was stronger in
191 extract fractions with higher procyanidin oligomers. Working with apple procyanidins, Sugiyama et al.
192 (2007) also described an inhibitory effect on pancreatic lipase activity which depended on size. Mao, Van
193 De Water, Keen, Schmitz, and Gershwin (2003) found that the effects on cytokine expression in
194 peripheral blood mononuclear cells depended on the cocoa procyanidin fraction evaluated. Faria et al.
195 (2006) showed that the simpler procyanidin structures, including catechin, have a higher antioxidant
196 activity, which correlates with their antiproliferative effect on the cell lines of breast cancer. Agarwal et
197 al. (2007) identified B2-3,3'-di-O-gallate as a major active constituent against growth inhibition and
198 apoptosis, relaying most of its power to its galloyl group because B2 was barely active. Similarly,
199 Lizarraga et al. (2007) observed that the fractions that were most efficient at inhibiting cell proliferation,
200 arresting the cell cycle in the G(2) phase and inducing apoptosis, were the grape fractions with the highest
201 percentage of galloylation and mean degree of polymerisation.

202 All these results together will be very helpful for understanding some of the differential and/or sometimes
203 contradictory effects described for complete extracts of natural origin. Simple procyanidin structures have
204 higher antioxidant properties. Short structures with higher galloylation seem to be more active as
205 antiproliferatives. At least one trimer and one dimer-gallate are needed to have metabolic effects.

206 ## 4. Conclusions

207 Monomeric structures and dimers (mainly fraction VI) of grape seed extract were the only effective as
208 anti-inflammatory agents. Procyanidin C1 was also very active as an anti-inflammatory compound.
209 Subfraction 12 was the most effective in all the parameters examined. As HPLC-ESI-MS and MALDI-
210 TOF analysis showed, this fraction contains a trimer (865) and a dimer-gallate (729). Therefore, a mix of
211 both molecules reproduces the bioactivity in glucose metabolism, lipid metabolism and macrophage

functionality which has previously been described for the total grape seed extract.

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Figure Captions

Fig. 1. Inhibition of PGE2 and NO production by C1 in LPS induced RAW 264.7 macrophages. RAW 264.7 macrophages were simultaneously stimulated with LPS for 19 h and incubated with C1 (trimeric procyanidin) at 5 mg/L and GSPE (grape seed procyanidin extract) at 45 mg/L as reference. PGE2 and NO production were measured after treatment. Results were normalised to control levels (100.0% ± 6.2). Each bar represents mean ± SEM of nine biological experiments. Different letters mean $p < 0.05$ as compared to GSPE treatment.

Fig. 2. Results from the second fractionation step. (a) Chromatograms of the most active subfractions. In correlative order: S2, S3, S4 and S12. (b) Stimulation of glucose uptake done by each of the subfractions obtained. After 2 h of depletion, differentiated 3T3-L1 adipocytes were treated for 30 min with 100 mg/L of total fraction VIII or each subfraction. Afterwards, glucose uptake was measured. Results are related to the maximum stimulation achieved by insulin (1.037 ± 0.023). (c and d) Effect of fraction VIII and its subfractions in de novo synthesised triacylglycerols (c) and total cholesterol (d) secretion to the extracellular media. HepG2 cultures were incubated for 6 h with fraction VIII or its subfractions at 25 mg/L and 14C-acetate. Afterwards, lipid fractions secreted to the cell culture media were quantified. Media lipid fraction levels (dpm/mg protein) were normalised to the control levels set at one. Each bar represents mean ± SEM. Letters above the bars mean $p < 0.05$ as compared to control. Different letters mean $p < 0.05$ as compared to fraction VIII treatment.

Tables

Table 1

Summary of the bioactivity of the monomeric pure forms. Values in italics mean a statistically significant positive effect ($p < 0.05$).

Monomeric structures	**Stimulation of glucose uptake (3T3-L1 adipocytes) 150 mg/L compound (stimulation vs insulin effect 1.04 ± 0.023)**	**PGE-2 production (RAW macrophages) 5 mg/L compound (% of inhibition vs LPS stimulation 100.0 ± 1.2)**	**NO production RAW macrophages 5 mg/L compound (% of inhibition vs LPS stimulation 100.0 ± 6.2)**
Vanillic acid	0.055 ± 0.018	*60.63 ± 9.61*	*69.69 ± 0.56*
Epicatechin	0.024 ± 0.014	*46.73 ± 0.73*	*64.77 ± 0.70*
Epicatechin gallate	NA	*48.18 ± 6.66*	*32.87 ± 8.72*
EGCG	NA	*53.72 ± 5.63*	*6.67 ± 3.56*
Catechin	0.051 ± 0.018	*57.98 ± 5.99*	103.7 ± 1.35
Gallic acid	0.048 ± 0.010	*46.63 ± 2.51*	*93.4 ± 0.8*
p-Hydroxiphenylacetic acid	0.054 ± 0.014	*55.31 ± 4.00*	111.79 ± 5.28

p-Coumaric	0.058 ± 0.020	NA	NA
3-Hydroxybenzoic acid	0.058 ± 0.008	NA	NA
Protocatequic acid	0.073 ± 0.049	NA	NA
Ferulic acid	0.092 ± 0.068	NA	NA
Hippuric acid	0.085 ± 0.047	NA	NA

327 Table 2

328 Summary of the bioactivity of the oligomeric pure compounds. Values in italics mean a statistically

329 significant positive effect ($p < 0.05$).

Oligomeric pure structures	**Stimulation of glucose uptake 150 mg/L compound (stimulation vs insulin effect 1.037 ± 0.023)**	**PGE-2 production 5 mg/L compound (% of inhibition vs LPS stimulation 100.0 ± 1.2)**	**NO production 5 mg/L compound (% of inhibition vs LPS stimulation 100.0 ± 6.2)**	**Total cholesterol secretion 25 mg/L compound (fold change vs control 1.00 ± 0.02)**	**Triacylglycerol secretion 25 mg/L compound (fold change vs control 1.01 ± 0.01)**
B3	0.09 ± 0.02	*69.92 ± 11.14*	*83.98 ± 4.56*	1.26 ± 0.16	1.18 ± 0.11

B1	0.08 ± 0.07	*70.78 ± 15.8*	*42.56 ± 3.52*	1.29 ± 0.12	1.05 ± 0.11
B2	0.09 ± 0.05	*79.86 ± 0.17*	*60.12 ± 7.49*	1.15 ± 0.11	1.07 ± 0.09
B4	0.07 ± 0.03	*68.92 ± 0.04*	*46.58 ± 3.90*	1.22 ± 0.13	1.20 ± 0.16
C1	*0.13 ± 0.00* (100 mg/L)	*44.38 ± 1.42*	*20.96 ± 5.85*	1.05 ± 0.11	*0.82 ± 0.04*

330 Table 3

331 Summary of the bioactivity of the fractions obtained from the grape seed-derived extract. Values in italics
332 mean a statistically significant positive effect ($p < 0.05$). In the first column, the intensity of the shade of
333 grey correlates to the increasing degree of polymerisation.

GSPE fractions (amount obtained)	**Stimulation glucose uptake 150 mg/L compound (vs insulin effect 1.04 ± 0.02)**	**PGE-2 production 5 mg/L compound (% of inhibition vs LPS stimulation 100.0 ± 1.2)**	**NO production 5 mg/L compound (% of inhibition vs LPS stimulation 100.0 ± 6.2)**	**Triacylglycerol secretion 25 mg/L compound (fold change vs control 1.01 ± 0.01)**	**Total cholesterol secretion 25 mg/L compound (fold change vs control 1.0 ± 0.02)**	**ApoB protein secretion 10 mg/L compound vs control 100.3 ± 0.3)**
I (25 mg)	-0.04 ± 0.01	NA	NA	1.07 ± 0.07	0.96 ± 0.06	NA

II (66 mg)	-0.05 ± 0.01	NA	NA	1.01 ± 0.12	1.03 ± 0.10	80.0 ± 12.8
III (6 mg)	-0.04 ± 0.02	NA	NA	0.95 ± 0.09	0.87 ± 0.08	*81.0 ± 3.9*
IV (4.5 mg)	-0.09 ± 0.09	51.91 ± 17.74	51.24 ± 14.63	0.85 ± 0.07	0.82 ± 0.05	NA
V (11.5 mg)	-0.02 ± 0.09	NA	52.56 ± 9.67	0.96 ± 0.06	1.03 ± 0.12	*78.5 ± 1.4*
VI (26 mg)	0.11 ± 0.03	*63.35 ± 14.12*	*23.03 ± 9.05*	0.83 ± 0.05	0.97 ± 0.11	79.3 ± 9.1
VII (18 mg)	*0.40 ± 0.03*	NA	*44.60 ± 8.39*	*0.43 ± 0.08*	*0.71 ± 0.18*	NA
VIII (33 mg)	*0.49 ± 0.02*	NA	*20.52 ± 5.85*	*0.36 ± 0.03*	*0.59 ± 0.05*	*46.0 ± 4.9*
X (23 mg)	*0.35 ± 0.04*	*63.34 ± 11.59*	*23.22 ± 5.76*	*0.32 ± 0.01*	*0.58 ± 0.06*	NA
XI (16 mg)	*0.40 ± 0.04*	NA	*48.73 ± 0.77*	*0.41 ± 0.05*	*0.77 ± 0.01*	NA
XII (15 mg)	NA	NA	*22.48 ± 5.67*	*0.36 ± 0.03*	*0.81 ± 0.03*	NA

XIII (75.4 mg)	NA	*53.85 ± 10.57*	*22.58 ± 3.96*	*0.45 ± 0.00*	*0.72 ± 0.03*	NA
XIV (81.6 mg)	NA	NA	*49.44 ± 1.90*	*0.34 ± 0.01*	*0.68 ± 0.06*	NA
XV (33.5 mg)	NA	NA	*26.58 ± 4.13*	*0.45 ± 0.03*	*0.77 ± 0.06*	NA

Figures

Figure 1

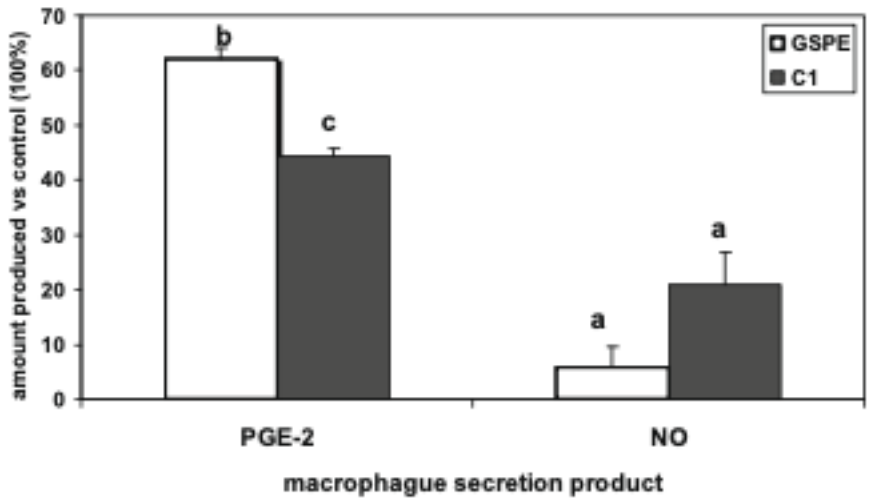
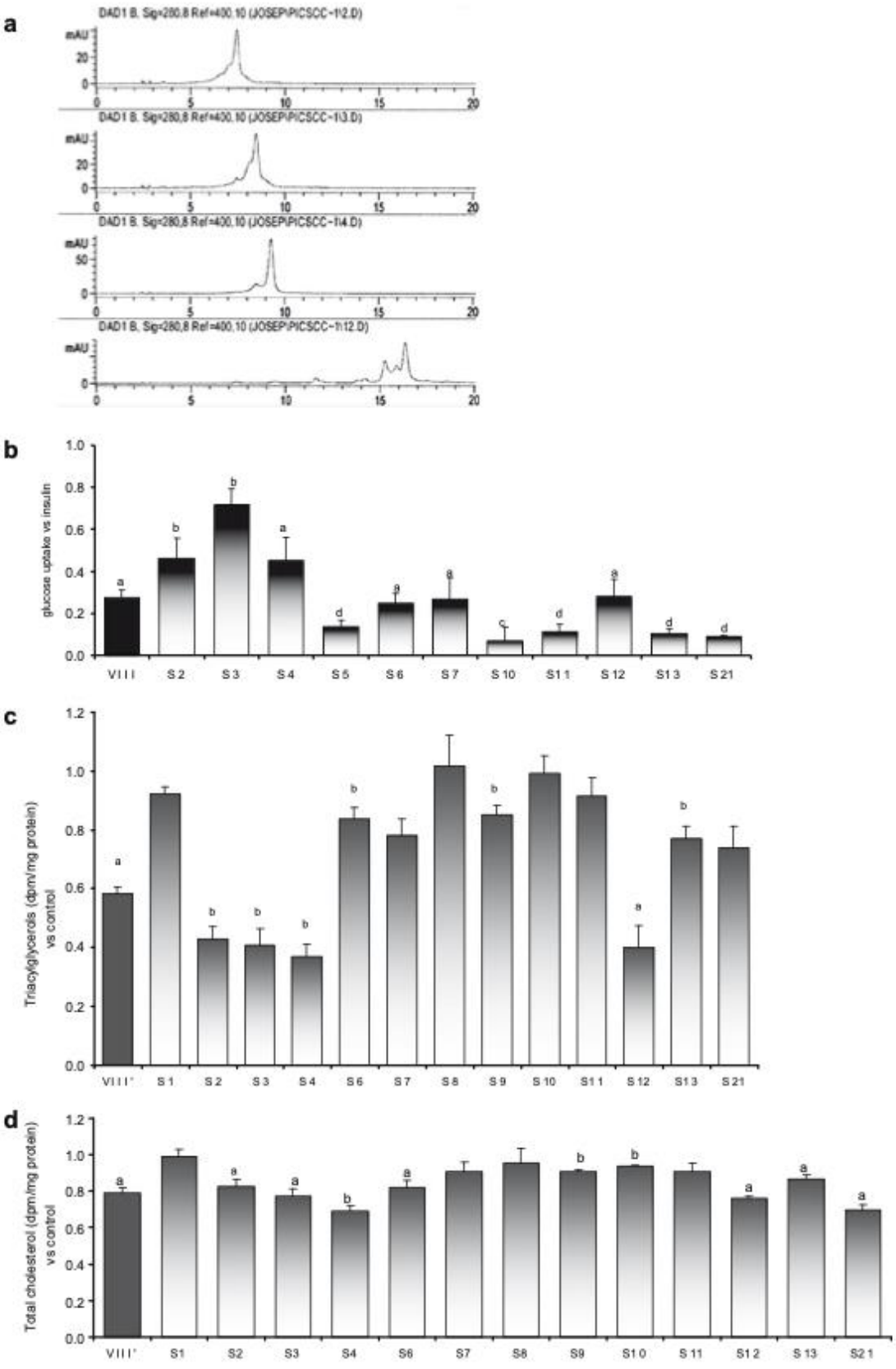


Figure 2


a
DAD1 B, Sig=280,8 Ref=400,10 (JOSEP\PICSCC~1\2.D)
DAD1 B, Sig=280,8 Ref=400,10 (JOSEP\PICSCC~1\3.D)
DAD1 B, Sig=280,8 Ref=400,10 (JOSEP\PICSCC~1\4.D)
DAD1 B, Sig=280,8 Ref=400,10 (JOSEP\PICSCC~1\12.D)
mAU
b
glucose uptake vs insulin
VIII S2 S3 S4 S5 S6 S7 S10 S11 S12 S13 S21
c
Triacylglycerols (dpm/mg protein) vs control
VIII* S1 S2 S3 S4 S6 S7 S8 S9 S10 S11 S12 S13 S21
d
Total cholesterol (dpm/mg protein) vs control
VIII* S1 S2 S3 S4 S6 S7 S8 S9 S10 S11 S12 S13 S21