

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

López-Chillón, MT; Carazo-Díaz, C; Prieto-Merino, D; Zafrilla, P; Moreno, DA; Villaño, D; (2018) Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects. *Clinical nutrition* (Edinburgh, Scotland). ISSN 0261-5614 DOI: <https://doi.org/10.1016/j.clnu.2018.03.006>

Downloaded from: <http://researchonline.lshtm.ac.uk/4647168/>

DOI: <https://doi.org/10.1016/j.clnu.2018.03.006>

Usage Guidelines:

Please refer to usage guidelines at <https://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by-nc-nd/2.5/>

<https://researchonline.lshtm.ac.uk>

Accepted Manuscript

Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects

M.T. López-Chillón, C. Carazo-Díaz, D. Prieto-Merino, P. Zafrilla, D.A. Moreno, D. Villaño



PII: S0261-5614(18)30118-3

DOI: [10.1016/j.clnu.2018.03.006](https://doi.org/10.1016/j.clnu.2018.03.006)

Reference: YCLNU 3420

To appear in: *Clinical Nutrition*

Received Date: 22 February 2018

Accepted Date: 6 March 2018

Please cite this article as: López-Chillón MT, Carazo-Díaz C, Prieto-Merino D, Zafrilla P, Moreno DA, Villaño D, Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects, *Clinical Nutrition* (2018), doi: 10.1016/j.clnu.2018.03.006.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Effects of long-term consumption of broccoli sprouts on inflammatory**
2 **markers in overweight subjects**

3
4 López-Chillón M.T.^a, Carazo-Díaz C.^b, Prieto-Merino D.^{bc}, Zafrilla P.^a, Moreno D.A.^{d ‡},
5 Villaño D.^a

6
7 ^a: Universidad Católica San Antonio de Murcia (UCAM), Department of Pharmacy,
8 Faculty of Health Sciences, Campus de los Jerónimos 30107 Guadalupe, Murcia, Spain

9 ^b Applied Statistical Methods in Medical Research Group, Catholic University of
10 Murcia (UCAM), Murcia, Spain

11 ^c Faculty of Epidemiology and Population Health, London School of Hygiene &
12 Tropical Medicine, London, United Kingdom

13 ^d CEBAS-CSIC, Department of Food Science and Technology, Phytochemistry Lab.
14 *Research Group on Quality, Safety and Bioactivity of Plant Foods*. Campus de
15 Espinardo - 25, E-30100 Espinardo, Murcia, Spain

16
17 ‡ Corresponding author: Diego A. Moreno. dmoreno@cebas.csic.es

18
19
20 **Keywords**

21 Glucosinolates; *Brassica oleracea*; Sulphoraphane; IL-6; Bioavailability; Inflammation

22

23 **ABSTRACT**

24 **Background & aims:** Broccoli sprouts represent an interesting choice of healthy food
25 product as they are rich in glucosinolates and their cognate bioactive metabolites,
26 isothiocyanates able to counteract the negative effects of diverse pathologies. As obesity
27 is linked to an inflammatory component, the aim of the study was to evaluate the anti-
28 inflammatory action of broccoli sprouts in overweight adult subjects.

29 **Methods:** An *in vivo* controlled study was performed in 40 healthy overweight subjects
30 (ClinicalTrials.gov ID NCT 03390855). Treatment phase consisted on the consumption
31 of broccoli sprouts (30 g/day) during 10 weeks and the follow-up phase of 10 weeks of
32 normal diet without consumption of these broccoli sprouts. Anthropometric parameters
33 as body fat mass, body weight, and BMI were determined. Inflammation status was
34 assessed by measuring levels of TNF- α , IL-6, IL-1 β and C-reactive protein.

35 **Results:** IL-6 levels significantly decreased (mean values from 4.76 pg/mL to 2.11
36 pg/mL with 70 days of broccoli consumption, $p < 0.001$) and during control phase the
37 inflammatory levels were maintained at low grade (mean values from 1.20 pg/mL to
38 2.66 pg/mL, $p < 0.001$). C-reactive protein significantly decreased as well.

39 **Conclusions:** This study represents an advance in intervention studies as the broccoli
40 sprouts were included in a daily dietary pattern in quantities that reflect a real
41 consumption. Further studies are necessary to elucidate the role of this healthy rich and
42 nutritious food product, but these promising results support the current evidence on the
43 healthy properties of *Brassica* varieties.

44

45 **INTRODUCTION**

46 Nowadays there is an increasing demand by consumers on healthy food products
47 prepared in convenient forms, simple to use and not containing additives. In this sense,
48 broccoli sprouts (*Brassica oleraceae var. italica*) are a good option, as they are the main
49 dietary source of glucosinolates, nitrogen-sulfur compounds, and phenolic derivatives
50 (flavonoid glycosides, and hydroxycinnamic acids), vitamins A, C, E, K and minerals
51 [1]. Glucosinolates are derivatives from amino acids and particularly abundant in
52 *Brassica* species and are believed to be responsible of the biological effects attributed to
53 these vegetables. Cultivar conditions can affect and improve the biosynthesis of these
54 secondary metabolites in the plant, favouring their storage in the sprouts. Broccoli
55 sprouts contain 10- to 50-fold higher concentrations of glucosinolates than the mature
56 broccoli [2].

57 Isothiocyanates are degradation products from glucosinolates formed by the hydrolytic
58 action of myrosinase vegetable enzyme when the vegetable structure is crushed, e.g.
59 during mastication process. Hydrolysis can also be performed by gastrointestinal
60 enzymes during digestion; the metabolic conversion of glucosinolates to isothiocyanates
61 is a crucial step for the benefits observed with the consumption of *Brassica* vegetables
62 [3]. Most of these reported effects are linked to anti-cancer properties and
63 epidemiological studies have evidenced a decrease in the risk of cancer with the intake
64 of cruciferous foods [4, 5]. Human clinical studies have mainly focused on these
65 antitumoral activities, with mechanisms including the upregulation of phase II
66 detoxification enzymes, as well as the direct action on cell cycle, causing apoptosis of
67 cancer cells [6] [7]. There is less evidence on the anti-inflammatory properties of
68 cruciferous vegetables in humans.

69 A non-communicable disease of high prevalence in Western societies is obesity.
70 Nowadays it is assumed that obesity is a condition characterized by a chronic low-grade
71 inflammation status. Levels of pro-inflammatory mediators as IL-6, TNF- α and C-
72 reactive protein are increased with the higher visceral adiposity and all of them are risk
73 factors for cardiovascular diseases, metabolic syndrome and diabetes [8]. The
74 permanent low-grade of inflammation is a response of the organism to the imbalance in
75 the nutrient due to energy excess and it is manifested in the development of insulin
76 resistance and type 2 diabetes [9]. Dietary interventions able to reduce the inflammatory
77 conditions linked to obesity may improve health conditions of overweight population.
78 The hypothesis of our research is that broccoli sprouts are able to reduce the
79 inflammatory status in overweight subjects due to their content in phytochemicals,
80 mainly glucosinolates. Hence, the aim of the study is to evaluate the changes in markers
81 of inflammation in overweight subjects after 10-week ingestion of broccoli sprouts as
82 well as to assess the bioavailability of glucosinolates to correlate the changes observed.

83

84 MATERIAL AND METHODS

85 Study design

86 We performed an interventional follow-up study to evaluate the effect of the daily
87 consumption of broccoli sprouts during 10 weeks (70 days).

88 The study was performed according with the Helsinki Declaration of Human Studies
89 and approved by the Ethical Committee of the Catholic University of Murcia as well as
90 the Bioethics Sub-Committee of the CSIC' Department of Ethics for the AGL-2013-
91 46247-P project registered also at ClinicalTrials.gov ID NCT 03390855. Volunteers
92 (n=40; 21 men, 19 women) were recruited in the Catholic University of Murcia
93 (UCAM) and all of them were informed on the characteristics of the study and they

94 signed the written-informed consent. The inclusion criteria were: BMI within the
95 overweight range according to the World Health Organization criteria (24.9-29.9
96 kg/m²), aged 35-55 years, taking no vitamins, supplements or medication during the
97 previous two months; no-smoking. The exclusion criteria were: diagnosed diseases as
98 hypertension and cardiovascular pathologies, diabetes, hepatic, gastrointestinal and
99 renal diseases, as well as the intake of drugs related to these pathologies, vegetarian
100 diet, pregnancy or breastfeeding. Dietetic and life style habits were recorded from all
101 participants.

102 There were no drop-outs during the whole period of study and no adverse effects were
103 reported due to the broccoli sprout ingestion. Physical parameters of the volunteer at the
104 beginning of the study are listed in **Table 1**.

105 One week before the beginning of the intervention period, subjects were asked to avoid
106 the consumption of *Brassica* vegetables (broccoli, radish, cauliflower, Brussel sprouts,
107 mustards, among others) and their derived products, and to follow a well-balanced diet
108 (based on Mediterranean diet), with no other food restriction criteria. These dietary
109 instructions were maintained during the entire period of study. Besides, they were
110 requested to record any sign of adverse effect, illness or deviation of the experimental
111 diet. The subjects maintained their usual lifestyles during the study.

112 On the first day, participants were given the portions of fresh broccoli sprouts to be
113 taken for the whole week (7 trays of broccoli sprouts of 30 g each) and each week they
114 had an appointment to provide them the fresh products. The intervention consisted on a
115 10-week period which included daily consumption of a portion (30 g) of raw, fresh
116 broccoli sprouts. This amount is consistent with a half- serving [11]. Subjects were
117 instructed to ingest 1 tray per day and to keep the trays refrigerated (4° C) at home. The
118 intake of the broccoli sprouts was included in their normal daily diet and no specific

119 time of consumption was established, with the only limitation of avoid cooking of the
120 sprouts and to consume them fresh. Cooking procedures can affect the content of
121 glucosinolates as well as their bioavailability [12, 13] and therefore some recipes were
122 provided to the participants to facilitate the intake of the sprouts without affecting the
123 phytochemical composition and absorption. We gave instructions to the volunteer of not
124 cooking broccoli sprouts but of consuming them in raw manner. They included the
125 sprouts in vegetable salads, cold pasta salads or in cold sandwiches with different
126 combinations of the following ingredients: cheese, ham, tomato, lettuce, grilled pork or
127 grilled vegetables (in both cases the sprouts should be added after the grill and once the
128 ingredient was lukewarm). It could also be included in burgers, as the “Californian
129 style burger”, which ingredients are broccoli sprouts, bacon, avocado, tomato, burger
130 and bread. Other recipes include elaboration of “Gazpacho” (Spanish cold soup made
131 with vegetables as tomato, cucumber, green pepper, onion, oil, vinegar and salt), or
132 mixed with smashed potatoes with melted cheese, or mixed with bread and spreadable
133 cheese. In all cases it was important to use in cold or warm temperatures so that the
134 glucosinolates were preserved.

135 After the intervention period, a follow-up recovery period for all subjects continued for
136 other 90 days with no ingestion of broccoli sprouts.

137 Fasting blood samples and 24-h urine samples were taken on day 0 (D0: just before
138 starting the intervention), day70 (D70: end of intervention period), day 90 (D90: 20
139 days after end of intervention) and 160 (D160: 90 days after end of intervention). Blood
140 samples were collected from each subject by venipuncture from the antecubital vein; 3
141 mL were placed in heparin tubes and centrifuged at 10000 rpm for 10 min at 4°C.
142 Plasma was aliquoted and stored at -80°C until analysis. Analysis were performed once
143 each period was finished and in the same batch to minimize analytical variations. The

144 total volume of the 24h-urine was recorded to calculate the absolute amounts of the
145 compounds and metabolites excreted in the study period and aliquots were frozen at -
146 80°C for further analysis. Body weight and percentage of fat mass were measured as
147 well and BMI calculated in each sampling time point.

148 **Broccoli sprouts**

149 Raw, fresh broccoli sprouts (*Brassica oleracea* var. *italica*) were supplied by
150 Aquaporins&Ingredients, S.L (Alcantarilla, Murcia, Spain). The sprouts were
151 biostimulated with methyl jasmonate 250 μ M, for 4 days previous to delivery, in order
152 to increase up to 2-fold levels the production of bioactive compounds, according to a
153 protocol previously validated [10]. In that study we performed some tests on elicitation
154 and seed priming to enrich the broccoli sprouts in glucosinolates. We used the elicitor
155 methyl jasmonate (MeJA) by priming the seeds as well as by spraying daily over the
156 cotyledons from day 4 to 7 of germination. We observed that MeJA at concentrations of
157 250 μ M act as stressor in the plant and enhances the biosynthesis of the phytochemicals
158 glucosinolates. Compared to control plants without MeJA treatment, the content of
159 compounds as the aliphatic glucosinolate glucoraphanin was enhanced up to a 70 % and
160 similar increases were observed with glucoiberin or glucobrassicin. In this way, we
161 improved the content of these health-promoting compounds. Other nutritional facts did
162 not change with these treatments.

163 Three trays of sprouts were collected once a week during the study, frozen and
164 lyophilized prior to analysis on glucosinolates and isothiocyanates, as previously
165 described [2]. The phytochemicals (glucosinolates) provided by the broccoli sprouts are
166 summarized in **Table 2**.

167 *Biochemical analysis*

168 Markers of inflammation as IL-6, C-reactive protein, IL-1 β and TNF- α in plasma were
169 determined in our laboratory using high-sensitivity ELISA kits. ichromaTM hsCRP kits
170 were purchased from Boditech Meed Inc.'s. Human IL-6 ELISA high sensitivity kits
171 were from BioVendor. Human IL-1 β high sensitivity ELISA kits and human TNF- α
172 high sensitivity ELISA kits were acquired from IBL International GmbH Instrumental.

173 *Analysis of glucosinolates and isothiocyanates in urine and plasma*

174 Levels of glucosinolates, isothiocyanates and their metabolites (GRA, IB, SFN, SFN-
175 GSH, SFN-NAC, SFN-CYS, I3C, 3,3-DIM) were measured in urine by a rapid,
176 sensitive and high throughput UHPLC-QqQ-MS/MS [14]. All LC-MS grade solvents
177 were obtained from J.T. Baker (Phillipsburg, New Jersey, USA). The standards of
178 Sulphoraphane, SFN-glutathione, SFN-cysteine and SFN-N-acetylcysteine (SFN, SFN-
179 GSH, SFN-CYS, SFN-NAC, respectively) and Iberin (IB), Indole-3-carbinol (I3C) and
180 3,3-Diindolyl-methane (DIM_3_3) were from SantaCruz Biotech (CA, USA).
181 Glucoraphanin (GRA) was obtained from Phytoplan (Diehm & Neuberger GmbH,
182 Heidelberg, Germany).

183 Urine and plasma samples were extracted using SPE Strata-X cartridges (33um
184 Polymeric Strong Cation) following manufacturer's instructions (Phenomenex,
185 Torrance, CA, USA). The cartridges were preconditioned with 2 mL of methanol and
186 equilibrated with 2 mL of water:formic acid (98:2, v/v). Samples (400 μ L) were diluted
187 with 2 mL water:formic acid (98:2, v/v) and loaded into the column. SPE cartridges
188 were washed with 2 mL water:formic acid (98:2, v/v). Elution of target metabolites was
189 performed with 1 mL of methanol/formic acid (98:2, v/v). Samples eluted were dried
190 using a SpeedVac concentrator (Savant SPD121P, Thermo Scientific, Massachusetts,
191 USA). The extracts were reconstituted in 200 μ L of mobile phases A/B (90:10, v/v) and
192 filtered with PTFE 0.22 μ m filters. Chromatographic separation was carried out using a

193 ZORBAX Eclipse Plus C-18 (2.1 x 50 mm, 1.8 μ m) (Agilent Technologies) and the
194 mobile phases employed were: solvent A ammonium acetate, 13 mM (pH 4 with acetic
195 acid) and solvent B acetonitrile/acetic acid (99.9:0.1 v/v) as previously described
196 [15]. Twenty microliters of each sample were acquired in a Agilent Technologies
197 UHPLC-1290 Series coupled to a 6460 QqQ-MS/MS (Agilent Technologies,
198 Waldbronn, Germany). Compounds were identified and quantified using MRM
199 transitions and positive or negative ESI mode for confirmation of the target analytes,
200 compared to available external standards [14]. Standard curves were prepared freshly
201 every day of analysis.

202 *Statistical analysis*

203 Continuous variables were summarised with means and standard deviations while
204 qualitative variables were summarised with proportions. We estimated the relative
205 change on the continuous biomarkers (weight, BMI, body fat, IL_6, C-reactive protein
206 and 3,3-DIM), between the different periods (before and after Broccoli ingestion) using
207 linear regression models. Additional models were run controlling for age and sex in
208 case these variables were confounding the effect of the broccoli. We then compared the
209 proportion of samples with detectable metabolites in sulphoraphane pathway (SFN,
210 SFN-CYS and SFN-NAC) between visits. We studied the association between changes
211 observed in body fat mass, IL6 and C-reactive protein (outcome variables) and changes
212 in metabolites 3,3-DIM, SFN-NAC, SFN-CYS and SFN (explanatory variables) using
213 linear regression models. Data were analysed using R (3.4.1. version) software package.

214 **RESULTS**

215 *Bioactive compounds in broccoli sprouts*

216 Broccoli sprouts from each of the 10 weeks of the study were characterized for their
217 glucosinolate (GLS) contents (**Table 2**). Results are presented as the serving portion

218 (30g) consumed daily by the volunteers. The major aliphatic glucosinolate in broccoli
219 sprout detected was glucoraphanin (GRA, 4-methyl-sulphinylbutyl glucosinolate) and
220 the major indolic glucosinolate detected was neoglucobrassicin (NGB, 1-methoxy3-
221 indolylmethyl glucosinolate). Total concentration of aliphatic glucosinolates was 80.50
222 mg/30 g f.w., equivalent to 6.22 $\mu\text{mol/g}$ fresh weight or 65.47 $\mu\text{mol/g}$ dry weight. This
223 concentration was two-fold higher than indolic glucosinolates (40.62 mg/30 g f.w.,
224 equivalent to 2.88 $\mu\text{mol/g}$ fresh weight or 30.32 $\mu\text{mol/g}$ dry weight). Volunteers
225 consumed an average of 51 mg (117 μmol) and 20 mg (42 μmol) of glucoraphanin and
226 neoglucobrassicin, respectively, on a daily basis, during the 70 days of the dietary
227 intervention.

228 *Biological effects of broccoli sprout on markers of inflammation and body composition*

229 Baseline characteristics of volunteer are described in **Table 1**. Changes on plasma
230 concentration of biomarkers at different time points of the intervention are shown in
231 **Table 3**. Day 0 and day 70 refers to the first and last days of the broccoli ingestion,
232 respectively. The days 90 and 160 refers to 20 days and 90 days of follow-up upon the
233 broccoli dosage period, respectively.

234 The evolution of mean values of continuous variables are shown in **Table 4**. The
235 metabolite 3,3'-diindolylmethane (3,3-DIM) was included in the statistical analysis as it
236 was detected in all volunteers, at concentrations higher than limit of quantification,
237 hence, for statistical purposes, it was treated as a continuous variable. Evolution of
238 ratios of continuous variables in each visit are shown in **Figure 1**.

239 No significant changes were observed in weight and BMI. By contrast, body fat mass
240 slightly decreased significantly after 70 days of broccoli consumption (ratio = 0.947, P-
241 value= 0.02586) and returned to basal levels at day 90, a state that was maintained until
242 day 160 (P-value= 0.94899 y P-value=0.07644).

243 Plasma interleukine-6 (IL-6) concentrations decreased significantly (by 38 %) after 70
244 days of broccoli ingestion as well, respect to basal value (ratio=0.381, P-value <
245 0.00001). Moreover, these lower levels continue to significantly decrease after 20 days
246 of ceasing broccoli ingestion (ratio = 0.195, P-value< 0.00001). At 90 days of the
247 follow up period (day 160), levels returned somewhat but without returning to baseline
248 values (ratio=0.472, P-value= 0.00000). **Figure 2** illustrates how the changes in IL-6,
249 during broccoli intake, depends on baseline values. The negative slope of the regression
250 line indicates that volunteer with higher concentrations at baseline tend to lose more
251 concentration of this biomarker. Decreases in C-reactive protein were also observed,
252 during broccoli ingestion period (ratio = 0.592, P-value= 0.00915). Shortly in the
253 follow-up period, levels returned to baseline conditions (P-value=0.92162 and P-
254 value=0.72756, at 90 and 160 days, respectively).

255 These results did not substantially change when we repeated the regression models
256 adjusting for age and sex. TNF α and IL-1 β were detected in a small number of samples
257 and most of them below the limit of quantification, hence no valid conclusions can be
258 inferred and data have not been considered for statistical purposes.

259 *Bioavailability and metabolism of glucosinolates and isothiocyanates*

260 Some glucosinolates and isothiocyanates, as glucoraphanin, glucoiberin, iberin,
261 glucoerucin, erucin and glucobrassicin were absent in the urine samples. Indole-3-
262 carbinol was detected only after broccoli ingestion in low quantities and in 50 % of
263 samples. In contrast, the metabolite 3,3'-diindolylmethane (3,3-DIM) was detected and
264 quantified in all volunteers and for statistical purposes it was treated as a continuous
265 variable. It increased significantly during broccoli ingestion (ratio = 1.947, P-value <
266 0.00001). Shortly in the follow-up period, levels returned to baseline conditions (P-
267 value=0.10484 and P-value=0.12312, at 90 and 160 days, respectively).

268 Metabolites from sulphoraphane pathway are present in 24 h-urine samples (**Table 3**);
269 the metabolite at higher amount was SFN-NAC (mean concentration 2.0301 μM ,
270 corresponding to 3.21 $\mu\text{mol}/24\text{ h}$), whereas SFN was the compound with the lowest
271 excretion (0.543 μM , corresponding to 0.77 $\mu\text{mol}/24\text{ h}$). The sum of SFN, SFN-NAC
272 and SFN-CYS was $\sim 5.11\ \mu\text{mol}/24\text{ h}$. Considering an amount of GRA of 117 μmol by
273 serving, a 4 % on average was metabolized through mercapturic acid pathway.

274 **Figure 3** shows the proportion of individuals in which the metabolites have been
275 detected and quantified at each visit and **Figure 4** shows the differences of these
276 proportions from baseline with their confidence intervals. The percentage of individuals
277 where SFN-NAC is detected increases significantly during broccoli intervention (45%
278 increase; P-value = 0.00001). Afterwards, the percentage diminishes although it is
279 statistically different from baseline (32.5 % difference; P-value = 0.00303). At 160
280 days, no significant differences from baseline are observed (P-value = 0.07139). Similar
281 behaviour is detected with SFN-CYS and SFN. Percentages increased during broccoli
282 ingestion (67.5 % increases in SFN-CYS P-value = 0.0000; 82.5 % increases in SFN; P-
283 value < 0.0001). Afterwards, the percentages of individuals detected decreased to
284 baseline conditions (P-value = 0.43858 and P-value 0.26355 for SFN-CYS and SFN,
285 respectively). This behaviour is maintained for the longer period at 160 days (P-value =
286 0.29330 and P-value 0.73532, respectively). SFN-GSH was detected in very few
287 samples of volunteer during broccoli ingestion (data not included), hence, it has not
288 been considered for statistical purposes.

289 The decrease in IL-6 levels was significantly related to the increase in 24 h-urine SFN
290 levels (p=0.03319). In case of C-reactive protein, the decrease was significantly related
291 to the increases in 24 h-urine SFN-NAC (p=0.04783) and SFN-CYS (p=0.04116).
292 (Supp. Table-6).

293

294

295 **DISCUSSION**

296 We conducted a human intervention study to test whether regular consumption of
297 broccoli sprouts improves inflammatory biomarkers in overweight subjects. Adipose
298 tissue is related to higher secretion of pro-inflammatory cytokines as TNF- α and IL-6
299 and elevated levels of these proteins have been described in overweight individuals [16,
300 17]. These proteins are linked to several disease states [18] and C-reactive is an
301 important predictive marker of cardiovascular events [19]; hence the reduction of their
302 levels with dietary intervention could contribute to a better prognosis on obesity-
303 associated disorders. In our study we observed a noticeable anti-inflammatory effect
304 with the ingestion of broccoli sprouts, with a significant reduction by 38 % and 59 % in
305 IL-6 and C-reactive protein concentrations, respectively.

306 Clinical studies on human participants on the anti-inflammatory properties of *Brassica*
307 products are scarce. Our research group has previously described a significant decrease
308 on markers of inflammatory processes, as the metabolites tetranor-PGEM (from
309 prostaglandins E₁ and E₂) and 11 β -PGF₂ α (from prostaglandin D₂) after consumption
310 of a single portion of broccoli sprouts [20]. Other authors have reported decreases on C-
311 reactive protein levels by 48 % after 10-day broccoli intake (250 g/day) in smokers,
312 confirming our results; however, no changes on IL-6 levels were detected [21].
313 Decreases in IL-6 and C-reactive protein were also observed after 14 days of
314 cruciferous consumption [22], but the amounts used (7g/kg body weight, 14 g/kg body
315 weight) far exceeded those of our experiment.

316 Differences in population studied, study design, type of *Brassica* or amount of product
317 consumed, could explain the different results observed. Our broccoli sprouts contained

318 significant quantities of aliphatic glucosinolates as glucoraphanin, glucoiberin and
319 glucoerucin, which derive from the aminoacid methionine, as well as indolic
320 glucosinolates as methoxy and hydroxy derivatives of glucobrassicin, that derive from
321 the aminoacid tryptophan. Broccoli sprouts are especially rich in glucoraphanin (up to
322 10-fold above adult organ (inflorescence) levels) that drop with the plant growing, as
323 the plant material increases without concomitant synthesis of glucoraphanin [23].
324 Hence, potential beneficial concentrations are easier to achieve with dietary quantities
325 of sprouts vs broccoli heads (inflorescences).

326 Levels of glucosinolates and their metabolites isothiocyanates were measured in 24h-
327 urine by UHPLC-MS/MS in order to ascertain the consumption of broccoli and with the
328 aim to find out if any metabolite is related to the changes in the biochemical parameters
329 observed. We did not observe significant levels of intact glucosinolates in 24 h-urine
330 samples, being explainable as these compounds suffer extensive modifications prior to
331 absorption in the gut. They are present in the intact plant as glucosides and, upon tissue
332 damage, the enzyme myrosinase catalyses their rapid hydrolysis of the glucose moiety
333 [24]. The aglycone of each glucosinolate suffers further hydrolytic metabolism to
334 isothiocyanate in the gastrointestinal tract by gut microbiota; these compounds are then
335 absorbed by enterocytes and distributed systemically [25]. In particular, the
336 isothiocyanate sulphoraphane (1-isothiocyanate-4-methyl-sulfinylbutane) is formed
337 from the glucosinolate glucoraphanin (4-methyl-sulphinylbutyl glucosinolate).
338 Therefore, isothiocyanates are the compounds mainly present in human tissues to which
339 can be attributed the biological activities.

340 In humans, the isothiocyanates are metabolized via the mercapturic acid pathway.
341 Conjugation with glutathione is catalysed by glutathione transferase and GSH-
342 conjugates are metabolized rendering SFN-CYS and SFN-NAC. It has been proven that

343 polymorphisms of these enzymes have a significant impact on ITC metabolism [26].
344 From the results described, the metabolites SFN-NAC, SFN-CYS and SFN can be
345 considered as good markers of ingestion, as their presence is related only with the
346 broccoli period, indicating the compliance of the experimental diet.

347 Concerning indole glucosinolates, indole-3-carbinol (I3C) is released by hydrolysis of
348 glucobrassicin (3-indolyl-methylglucosinolate) by myrosinase action. This type of
349 indole glucosinolates are present in seeds, mature plant and some sprouts cultivars, but
350 are not commonly present in all *Brassica* varieties [27, 28]. After ingestion, I3C is
351 modified by the acidic pH in the stomach and dimerizes to 3,3'-diindolymethane (3,3-
352 DIM) [29]. Hence, the presence of 3,3-DIM in the 24 h-urine samples is related to the
353 metabolism of glucobrassicin derivatives present in our broccoli sprouts.

354 The increases on broccoli metabolites were significantly related to the decreases in IL-6
355 and C-reactive protein levels, what suggests their implication in the modulation of these
356 pro-inflammatory proteins. Studies on cellular models have shown that the mechanism
357 of interaction is common in isothiocyanates and similar to that of endogenous hormones
358 as steroids or vitamins A and D. This type of compounds possesses electrophile groups
359 that interact with nucleophilic moieties of transcription factors, down- or up-regulating
360 their activity [30]; it has been shown that sulphoraphane interacts with the redox-
361 sensitive transcription factor Nrf2, to permit its translocation into the nucleus, where it
362 binds to the antioxidant response element (ARE) and activates the synthesis of proteins
363 related to the response to stress, as phase II detoxification enzymes and quinone
364 reductases [31-34]. Besides, SFN inhibits activation of NF- κ B, a central transcription
365 factor in inflammation process and the gene expression of proinflammatory mediators
366 [34, 35]. This signalling pathway is redox sensitive as depends on the balance between
367 ROS intracellular concentration and GSH levels. Changes on GSH levels by SFN may

368 influence in this anti-inflammatory action. Other authors observed an anti-inflammatory
369 effect by induction of Nrf2-pathway of broccoli sprout extract in human skin [36] and
370 nasal lavage cells [32] in healthy subjects as well as in patients with chronic obstructive
371 pulmonary disease (COPD) [37].

372 3,3'-DIM has shown to reduce transcriptional activity of NF- κ B, what results in lower
373 levels of inflammatory mediators as IL-6, in activated macrophages [38] as well in
374 different models of inflammation in mice [39, 40]. It has been pointed out the possible
375 synergistic interaction of both SFN and 3,3'-DIM [7] and the isothiocyanates erucin and
376 sulphoraphane are interconvertible [41], so that the anti-inflammatory effects observed
377 with broccoli sprouts intake are likely due to the combined effects of all the hydrolysis
378 products of glucosinolates.

379 Concerning anthropometric parameters, after 10 week of the daily consumption of
380 broccoli sprouts, weight and body mass index were not altered; however, body fat mass
381 significantly decreased with broccoli intervention. It has been described that the
382 metabolite I3C decreases adipogenesis by supressing pathways of lipid accumulation
383 mediated by PPAR γ [42]; however, we did not detect I3C in 24 h-urine as it is mainly
384 excreted in its metabolite 3,3'-DIM. We did not observe a significant correlation
385 between the increase in 3,3'-DIM and the decrease in fat mass; no further experiments
386 were performed to corroborate an additional hypothesis about the effects on
387 adipogenesis.

388 A limitation of this study is the lack of a parallel randomised control group which would
389 be ideal to stablish causality links between broccoli intakes and the change in biomarker
390 levels. The post-intervention follow up is not an ideal control period as several other
391 factors might have changes in the individuals or the environment. However, the strong
392 changes observed in the inflammatory markers at the end of the intervention and their

393 recuperation afterwards could be an indication of the beneficial effect of the broccoli
394 that will have to be tested in a proper trial. On the other hand, overweight is frequently
395 associated with other pathologies as hypertension, cardiovascular events, insulin
396 resistance or type 2 diabetes and, due to the complex interactions among them, we
397 limited the study to people with overweight status according to WHO criteria, but
398 without any pathology or clinical disorder. Hence, our result can only be extrapolated to
399 these type of population and not to the overall that could include people with some
400 concomitant pathologies.

401

402

403 **CONCLUSIONS**

404 The consumption of broccoli sprouts in a real dietary serving is able to affect IL-6 and
405 C-reactive protein levels in overweight subjects, hence attenuating chronic
406 inflammation. Further research with broccoli sprouts including other biomarkers and
407 mechanistic studies are necessary to elucidate the role of this healthy rich and nutritious
408 food product, but these promising results support the current evidence on the properties
409 of this *Brassica* specie for disease prevention.

410 **ACKNOWLEDGMENTS**

411 Authors would like to thank the support and collaboration of Aquaporins & Ingredients
412 S.L. specially from their technical department assistance and their facilities for the
413 production of the broccoli sprouts needed in the clinical study according to the
414 controlled certified conditions of the Ecological Agriculture Council of Murcia Region
415 (CAERM) reference ES-ECO-024-MU, to guarantee appropriate standards of quality
416 and safety for consumption.

417 **STATEMENT OF AUTHORSHIP**

418 López-Chillón MT carried out data analyses and contributed to the interpretation of the
419 findings; Carazo-Díaz MC and Prieto-Merino D performed the statistical analysis;
420 Zafrilla P contributed to data analysis and discussion of the manuscript; Moreno D.A.
421 Principal Investigator and general management of the AGL-2013-46247-P project,
422 contributed with the funding the study, design of experiment, discussion and writing of
423 manuscript. Villaño D contributed to the interpretation of analyses and discussion of the
424 manuscript, statistical management of data and writing of manuscript.

425 **CONFLICT OF INTEREST STATEMENT AND FUNDING SOURCES**

426 All Co-authors declare no conflicts of interest.

427 This work was supported by the Spanish Ministry of Economy, Industry and
428 Competitiveness (MINECO) and European Regional Development Fund (ERDF)
429 through Research Project AGL2013-46247-P, and the Grant for Research Groups of
430 Excellence from the Murcia Regional Agency for Science and Technology (Fundación
431 Séneca), Project 19900/GERM/15.

432 **REFERENCES**

- 433 [1] West, L. G., Meyer, K. A., Balch, B. A., Rossi, F. J., *et al.*, Glucoraphanin and 4-
434 hydroxyglucobrassicin contents in seeds of 59 cultivars of broccoli, raab, kohlrabi,
435 radish, cauliflower, brussels sprouts, kale, and cabbage. *Journal Of Agricultural And*
436 *Food Chemistry* 2004, 52, 916-926.
- 437 [2] Baenas, N., García-Viguera, C., Moreno, D. A., Elicitation: a tool for enriching the
438 bioactive composition of foods. *Molecules (Basel, Switzerland)* 2014, 19, 13541-13563.
- 439 [3] Dinkova-Kostova, A. T., Kostov, R. V., Glucosinolates and isothiocyanates in health
440 and disease. *Trends in Molecular Medicine* 2012, 18, 337-347.

- 441 [4] Wu, Q.-J., Yang, Y., Wang, J., Han, L.-H., Xiang, Y.-B., Cruciferous vegetable
442 consumption and gastric cancer risk: a meta-analysis of epidemiological studies. *Cancer*
443 *Science* 2013, *104*, 1067-1073.
- 444 [5] Hu, J., Hu, Y., Hu, Y., Zheng, S., Intake of cruciferous vegetables is associated with
445 reduced risk of ovarian cancer: a meta-analysis. *Asia Pacific Journal of Clinical*
446 *Nutrition* 2015, *24*, 101-109.
- 447 [6] Abdull Razis, A., Nicola, G., Pagnotta, E., Iori, R., Ioannides, C., 4-Methylsulfanyl-
448 3-butenyl isothiocyanate derived from glucoraphasatin is a potent inducer of rat hepatic
449 phase II enzymes and a potential chemopreventive agent. *Archives of Toxicology* 2012,
450 *86*, 183-194.
- 451 [7] Jeffery, E. H., Araya, M., Physiological effects of broccoli consumption.
452 *Phytochemistry Reviews* 2009, *8*, 283-298.
- 453 [8] Moschen, A. R., Molnar, C., Enrich, B., Geiger, S., *et al.*, Adipose and liver
454 expression of interleukin (IL)-1 family members in morbid obesity and effects of weight
455 loss. *Molecular Medicine (Cambridge, Mass.)* 2011, *17*, 840-845.
- 456 [9] Lumeng, C. N., Saltiel, A. R., Inflammatory links between obesity and metabolic
457 disease. *Journal of Clinical Investigation* 2011, *121*, 2111-2117.
- 458 [10] Baenas, N., Villaño, D., García-Viguera, C., Moreno, D. A., Optimizing elicitation
459 and seed priming to enrich broccoli and radish sprouts in glucosinolates. *Food*
460 *Chemistry* 2016, *204*, 314-319.
- 461 [11] Services, F. a. D. A. F. U. D. o. H. a. H., 2001.
- 462 [12] Palermo, M., Pellegrini, N., Fogliano, V., The effect of cooking on the
463 phytochemical content of vegetables. *Journal Of The Science Of Food And Agriculture*
464 2014, *94*, 1057-1070.

- 465 [13] Vermeulen, M., Klöpping-Ketelaars, I. W. A. A., van den Berg, R., Vaes, W. H. J.,
466 Bioavailability and kinetics of sulforaphane in humans after consumption of cooked
467 versus raw broccoli. *Journal Of Agricultural And Food Chemistry* 2008, *56*, 10505-
468 10509.
- 469 [14] Baenas, N., Suárez-Martínez, C., García-Viguera, C., Moreno, D. A.,
470 Bioavailability and new biomarkers of cruciferous sprouts consumption. *Food Research*
471 *International (Ottawa, Ont.)* 2017, *100*, 497-503.
- 472 [15] Dominguez-Perles, R., Medina, S., Moreno, D. Á., García-Viguera, C., *et al.*, A
473 new ultra-rapid UHPLC/MS/MS method for assessing glucoraphanin and sulforaphane
474 bioavailability in human urine. *Food Chemistry* 2014, *143*, 132-138.
- 475 [16] Kang, Y. E., Kim, J. M., Joung, K. H., Lee, J. H., *et al.*, The Roles of Adipokines,
476 Proinflammatory Cytokines, and Adipose Tissue Macrophages in Obesity-Associated
477 Insulin Resistance in Modest Obesity and Early Metabolic Dysfunction. *Plos One* 2016,
478 *11*, e0154003-e0154003.
- 479 [17] Jorge, A. S. B., Jorge, G. C. B., Paraíso, A. F., Franco, R. M. P., *et al.*, Brown and
480 White Adipose Tissue Expression of IL6, UCP1 and SIRT1 are Associated with
481 Alterations in Clinical, Metabolic and Anthropometric Parameters in Obese Humans.
482 *Experimental & Clinical Endocrinology & Diabetes* 2017, *125*, 163-170.
- 483 [18] Bienvenu, J., Monneret, G., Fabien, N., Revillard, J. P., The clinical usefulness of
484 the measurement of cytokines. *Clinical Chemistry And Laboratory Medicine* 2000, *38*,
485 267-285.
- 486 [19] Whelton, S. P., Roy, P., Astor, B. C., Zhang, L., *et al.*, Elevated High-Sensitivity
487 C-Reactive Protein as a Risk Marker of the Attenuated Relationship Between Serum
488 Cholesterol and Cardiovascular Events at Older Age. *American Journal of*
489 *Epidemiology* 2013, *178*, 1076-1084.

- 490 [20] Medina, S., Domínguez-Perles, R., Moreno, D. A., García-Viguera, C., *et al.*, The
491 intake of broccoli sprouts modulates the inflammatory and vascular prostanoids but not
492 the oxidative stress-related isoprostanes in healthy humans. *Food Chemistry* 2015, *173*,
493 1187-1194.
- 494 [21] Riso, P., Vendrame, S., Del Bo, C., Martini, D., *et al.*, Effect of 10-day broccoli
495 consumption on inflammatory status of young healthy smokers. *International Journal*
496 *Of Food Sciences And Nutrition* 2014, *65*, 106-111.
- 497 [22] Navarro, S. L., Schwarz, Y., Song, X., Wang, C.-Y., *et al.*, Cruciferous vegetables
498 have variable effects on biomarkers of systemic inflammation in a randomized
499 controlled trial in healthy young adults. *Journal of Nutrition* 2014, *144*, 1850-1857.
- 500 [23] Fahey, J. W., Yuesheng, Z., Broccoli sprouts: An exceptionally rich source of
501 inducers of enzymes that protect against. *Proceedings of the National Academy of*
502 *Sciences of the United States of America* 1997, *94*, 10367.
- 503 [24] Bones, A. M., Rossiter, J. T., The enzymic and chemically induced decomposition
504 of glucosinolates. *Phytochemistry* 2006, *67*, 1053-1067.
- 505 [25] Angelino, D., Jeffery, E., Glucosinolate hydrolysis and bioavailability of resulting
506 isothiocyanates: Focus on glucoraphanin. *Journal of Functional Foods* 2014, *7*, 67-76.
- 507 [26] Joseph, M. A., Moysich, K. B., Freudenheim, J. L., Shields, P. G., *et al.*,
508 Cruciferous vegetables, genetic polymorphisms in glutathione S-transferases M1 and
509 T1, and prostate cancer risk. *Nutrition And Cancer* 2004, *50*, 206-213.
- 510 [27] Higdon, J. V., Delage, B., Williams, D. E., Dashwood, R. H., Cruciferous
511 vegetables and human cancer risk: epidemiologic evidence and mechanistic basis.
512 *Pharmacological Research* 2007, *55*, 224-236.

- 513 [28] Sayeed, M. A., Bracci, M., Lazzarini, R., Tomasetti, M., *et al.*, Use of potential
514 dietary phytochemicals to target miRNA: Promising option for breast cancer prevention
515 and treatment? *Journal of Functional Foods* 2017, 28, 177-193.
- 516 [29] Fujioka, N., Ainslie-Waldman, C. E., Upadhyaya, P., Carmella, S. G., *et al.*,
517 Urinary 3,3'-Diindolylmethane: A Biomarker of Glucobrassicin Exposure and Indole-3-
518 Carbinol Uptake in Humans. *Cancer Epidemiology, Biomarkers & Prevention* 2014, 23,
519 282-287.
- 520 [30] Houghton, C. A., Fassett, R. G., Coombes, J. S., Sulforaphane and Other
521 Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the
522 Reality? *Oxidative Medicine And Cellular Longevity* 2016, 2016, 7857186-7857186.
- 523 [31] Hu, C., Egger, A. L., Mesecar, A. D., van Breemen, R. B., Modification of keap1
524 cysteine residues by sulforaphane. *Chemical Research In Toxicology* 2011, 24, 515-521.
- 525 [32] Riedl, M. A., Saxon, A., Diaz-Sanchez, D., Oral sulforaphane increases Phase II
526 antioxidant enzymes in the human upper airway. *Clinical Immunology (Orlando, Fla.)*
527 2009, 130, 244-251.
- 528 [33] Wagner, A. E., Boesch-Saadatmandi, C., Dose, J., Schultheiss, G., Rimbach, G.,
529 Anti-inflammatory potential of allyl-isothiocyanate--role of Nrf2, NF-(κ) B and
530 microRNA-155. *Journal Of Cellular And Molecular Medicine* 2012, 16, 836-843.
- 531 [34] Sturm, C., Wagner, A. E., Brassica-Derived Plant Bioactives as Modulators of
532 Chemopreventive and Inflammatory Signaling Pathways. *International Journal Of*
533 *Molecular Sciences* 2017, 18.
- 534 [35] Heiss, E., Herhaus, C., Klimo, K., Bartsch, H., Gerhäuser, C., Nuclear factor kappa
535 B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *The*
536 *Journal Of Biological Chemistry* 2001, 276, 32008-32015.

- 537 [36] Dinkova-Kostova, A. T., Fahey, J. W., Wade, K. L., Jenkins, S. N., *et al.*, Induction
538 of the phase 2 response in mouse and human skin by sulforaphane-containing broccoli
539 sprout extracts. *Cancer Epidemiology, Biomarkers & Prevention: A Publication Of The*
540 *American Association For Cancer Research, Cosponsored By The American Society Of*
541 *Preventive Oncology* 2007, *16*, 847-851.
- 542 [37] Harvey, C. J., Thimmulappa, R. K., Sethi, S., Kong, X., *et al.*, Targeting Nrf2
543 signaling improves bacterial clearance by alveolar macrophages in patients with COPD
544 and in a mouse model. *Science Translational Medicine* 2011, *3*, 78ra32-78ra32.
- 545 [38] Cho, H. J., Seon, M. R., Lee, Y. M., Kim, J., *et al.*, 3,3'-Diindolylmethane
546 suppresses the inflammatory response to lipopolysaccharide in murine macrophages.
547 *Journal of Nutrition* 2008, *138*, 17-23.
- 548 [39] Jeon, E.-J., Davaatseren, M., Hwang, J.-T., Park, J. H., *et al.*, Effect of Oral
549 Administration of 3,3'-Diindolylmethane on Dextran Sodium Sulfate-Induced Acute
550 Colitis in Mice. *Journal Of Agricultural And Food Chemistry* 2016.
- 551 [40] Kim, Y. H., Kwon, H.-S., Kim, D. H., Shin, E. K., *et al.*, 3,3'-diindolylmethane
552 attenuates colonic inflammation and tumorigenesis in mice. *Inflammatory Bowel*
553 *Diseases* 2009, *15*, 1164-1173.
- 554 [41] Clarke, J. D., Hsu, A., Riedl, K., Bella, D., *et al.*, Bioavailability and inter-
555 conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or
556 broccoli supplement in a cross-over study design. *Pharmacological Research* 2011, *64*,
557 456-463.
- 558 [42] Choi, Y., Kim, Y., Park, S., Lee, K. W., Park, T., Indole-3-carbinol prevents diet-
559 induced obesity through modulation of multiple genes related to adipogenesis,
560 thermogenesis or inflammation in the visceral adipose tissue of mice. *The Journal Of*
561 *Nutritional Biochemistry* 2012, *23*, 1732-1739.

562

563 **FIGURE LEGENDS**

564 Figure 1. Evolution of ratios of continuous variables in each visit according to model
565 (1): a) changes in weight, BMI and body fat mass; b) changes in IL-6 and C-reactive
566 protein levels

567 Figure 2. Example of how changes in IL-6 during broccoli intake depends on baseline
568 values of IL-6 (variables log-transformed).

569 Figure 3. Proportion of individuals and 95 % IC of metabolites detected at each visit

570 Figure 4. Changes in binary variables over periods

571

Table 1. Baseline characteristics of volunteer (n=40; 21 men, 19 women)

Variable	Mean \pm standard deviation
Age (years)	46 \pm 6
Height (m)	1.72 \pm 0.08
Weight (kg)	85.8 \pm 16.7
BMI (kg/m ²)	28.9 \pm 4.0
Body fat mass (%)	30.34 \pm 7.54

Table 2. Glucosinolate contents in broccoli sprouts daily portions (mg/30 g F.W)

	Mean \pm Standard deviation (n=3)
Gluciberin (GIB)	19.28 \pm 0.98
Glucoraphanin (GRA)	51.08 \pm 1.06
4-Hydroxyglucobrassicin (HGB)	3.67 \pm 0.41
Glucoerucin (GER)	10.14 \pm 1.20
Glucobrassicin (GBS)	9.69 \pm 0.95
4-Methoxyglucobrassicin (MBG)	7.14 \pm 0.61
Neoglucobrassicin (NBG)	20.11 \pm 1.66
Aliphatic Glucosinolates (Σ)	80.50 \pm 2.18
Indolic Glucosinolates (Σ)	40.62 \pm 2.07
Total (Σ)	121.11 \pm 4.00

Table 3. Changes observed with broccoli treatment as well as during follow-up period.

Values are expressed as mean (confidence interval 95 %)

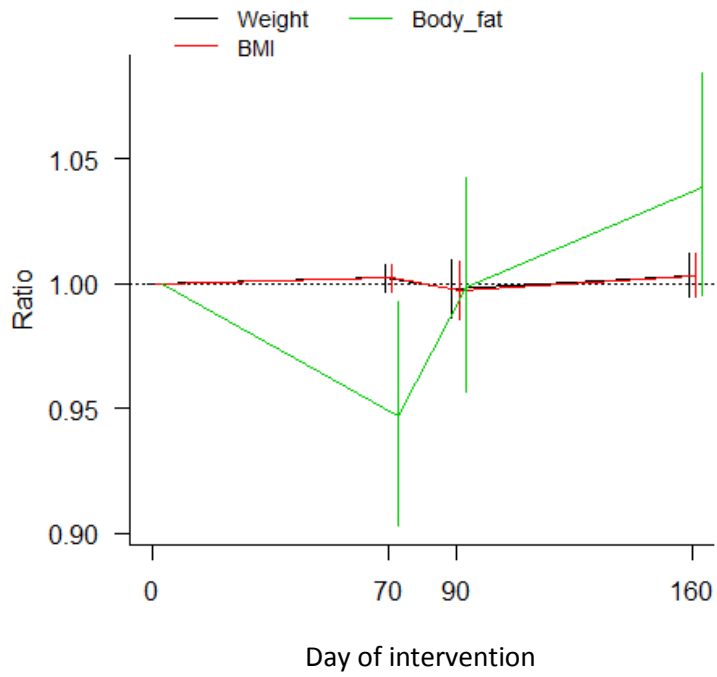
Variable	Day 0	Day 70	Day 90	Day 160
Weight (kg)	85.79 (80.38–91.20)	85.69 (80.42–90.95)	83.83 (79.47–88.18)	84.04 (79.66–88.41)
BMI (kg/m ²)	28.88 (27.56–30.20)	28.93 (27.63–30.23)	28.49 (27.40–29.57)	28.60 (27.52–29.68)
Body fat mass (%)	30.34 (27.29–33.39)	29.32 (26.93–31.71)	30.29 (27.87–32.72)	32.09 (29.69–34.49)
IL-6 (pg/mL)	4.76 (4.21–5.31)	2.11 (1.61–2.61)	1.20 (0.88–1.52)	2.66 (1.89–3.44)
C-reactive protein (µg/mL)	2.42 (1.45–3.40)	1.52 (0.70–2.34)	1.92 (1.02–2.82)	2.32 (1.07–3.56)
SFN-NAC (µM)	0.193 (0.00–0.41)	2.301 (1.85–2.75)	0.023 (0.01–0.04)	0.094 (0.00–0.19)
SFN-CYS (µM)	0.116 (0.00–0.26)	0.800 (0.57–1.03)	0.078 (0.00–0.22)	0.081 (0.00–0.19)
3,3-DIM (µM)	0.484 (0.38–0.59)	0.707 (0.61–0.80)	0.449 (0.33–0.57)	0.461 (0.36–0.56)
SFN (µM)	0.098 (0.00–0.23)	0.543 (0.40–0.69)	0.038 (0.00–0.13)	0.022 (0.01–0.03)

Table 4. Evolution of mean values (ratios) on the time points *

Variable	Mean	Ratio	95% CI	P-value
Weight (kg)	86.141			
From D0 to D70	86.346	1.002	(0.997 to 1.008)	0.38388
From D0 to D90	85.983	0.998	(0.987 to 1.010)	0.74963
From D0 to D160	86.424	1.003	(0.994 to 1.012)	0.45589
Body mass index (kg/m ²)	28.877			
From D0 to D70	28.945	1.002	(0.997 to 1.008)	0.39007
From D0 to D90	28.797	0.997	(0.986 to 1.009)	0.62441
From D0 to D160	28.971	1.003	(0.994 to 1.012)	0.46125
Body fat mass (%)	28.834			
From D0 to D70	27.298	0.947	(0.903 to 0.993)	0.02586
From D0 to D90	28.795	0.999	(0.956 to 1.043)	0.94899
From D0 to D160	29.955	1.039	(0.996 to 1.084)	0.07644
IL_6 (pg/mL)	4.594			
From D0 to D70	1.748	0.381	(0.298 to 0.486)	<0.00001
From D0 to D90	0.896	0.195	(0.149 to 0.255)	<0.00001
From D0 to D160	2.170	0.472	(0.366 to 0.609)	<0.00001
C-reactive protein (µg/mL)	1.431			
From D0 to D70	0.847	0.592	(0.405 to 0.865)	0.00915
From D0 to D90	1.459	1.020	(0.677 to 1.536)	0.92162
From D0 to D160	1.553	1.085	(0.665 to 1.771)	0.72756
DIM_3_3 (µM)	0.334			
From D0 to D70	0.650	1.947	(1.705 to 2.223)	<0.00001
From D0 to D90	0.335	0.757	(0.539 to 1.063)	0.10484
From D0 to D160	0.376	0.850	(0.689 to 1.048)	0.12312

*: data are adjusted by baseline levels

a)



b)

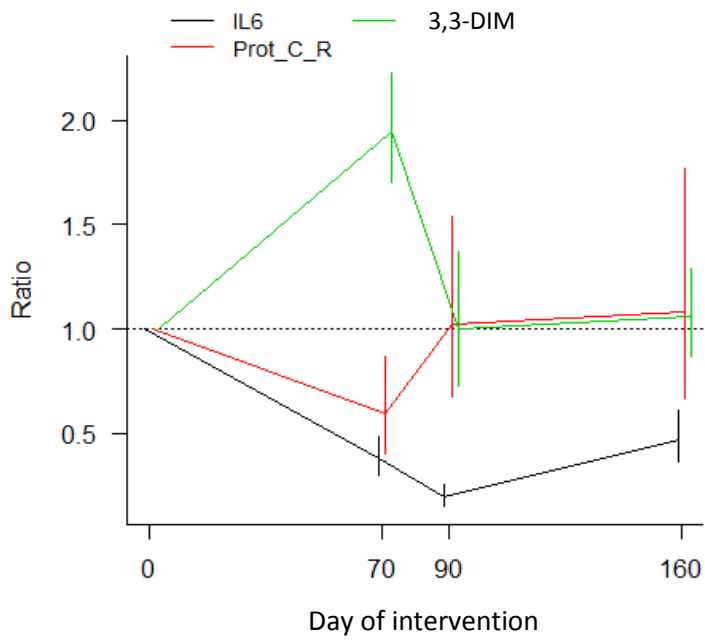


Figure 1.

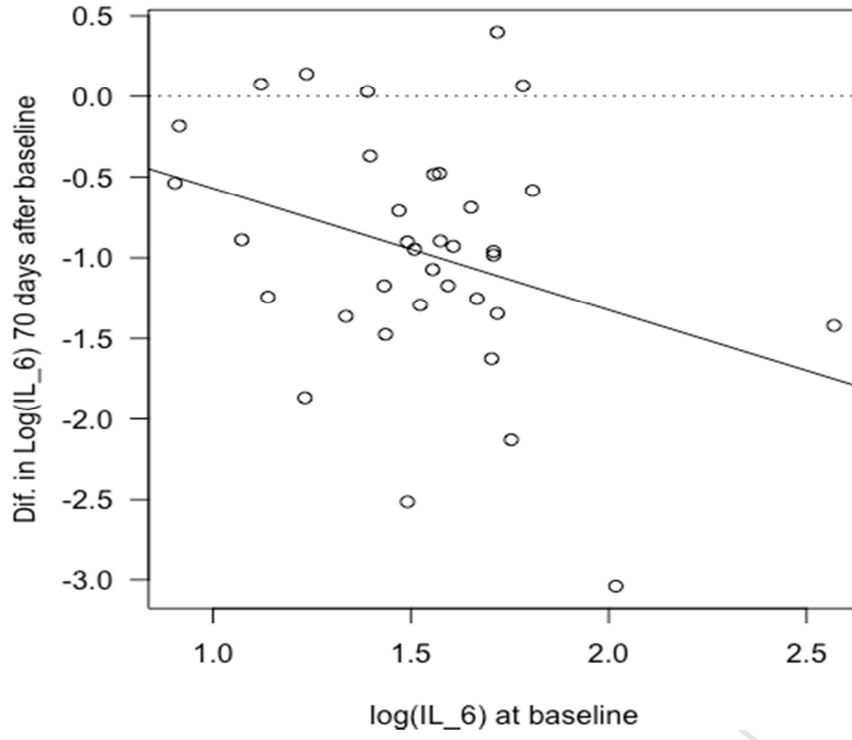


Figure 2.

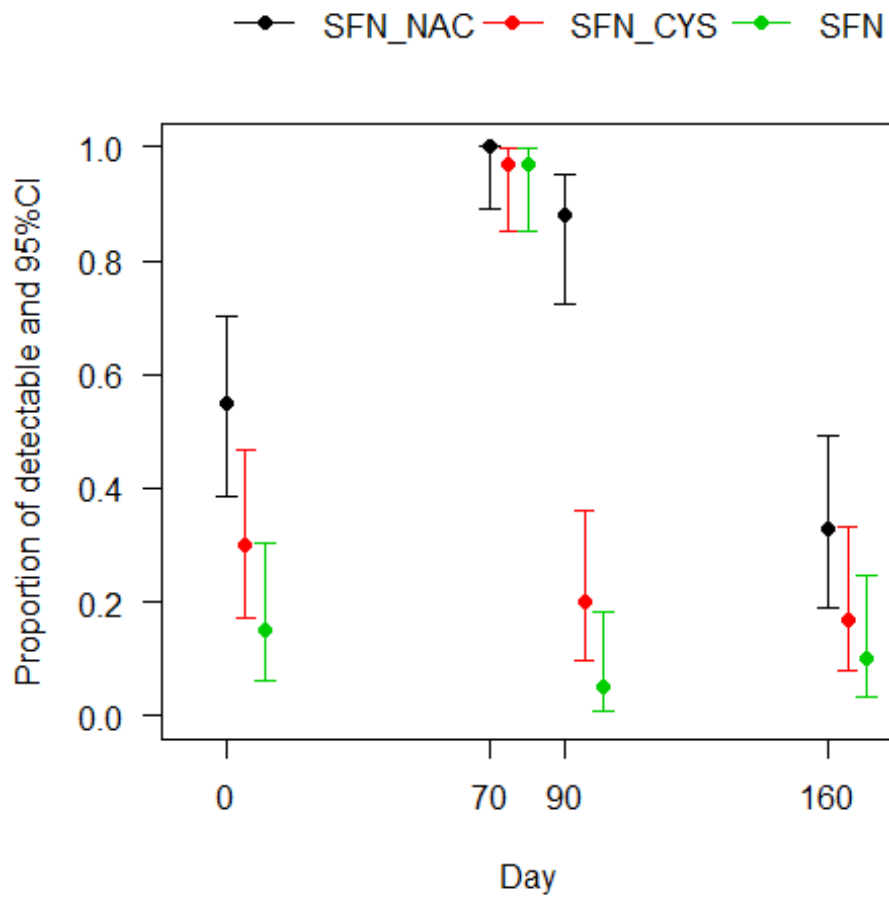


Figure 3.

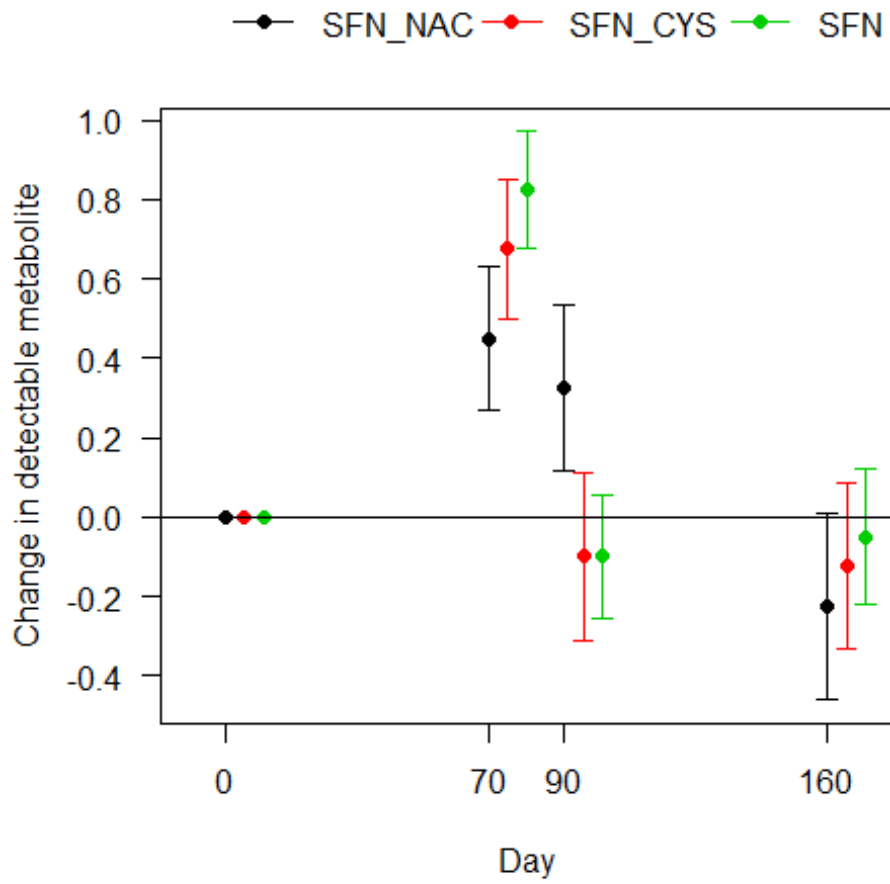


Figure 4.