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# Proceedings of the Nutrition Society



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## The role of bioactives in energy metabolism and metabolic syndrome

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**1    The role of bioactives in energy metabolism and metabolic syndrome**

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17          Short title: Bioactives in the management of MetS

18

**19    Abstract**

20          Some food bioactives potentially exert anti-obesity effects. Anthocyanins, catechins, beta-glucan, and  
21          n-3 long chain polyunsaturated fatty acids are among the most promising candidates and have been  
22          considered as a strategy for the development of functional foods counteracting body weight gain. At  
23          present, clinical trials, reviews and meta-analyses addressing anti-obesity effects of various bioactives  
24          or bioactive-rich foods show contradictory results. Abdominal obesity is an important criterion for  
25          metabolic syndrome diagnosis along with glucose intolerance, dyslipidemia, and hypertension. Food  
26          bioactives are supposed to exert beneficial effects on these parameters, therefore representing an  
27          alternative therapy approaches for the treatment of the metabolic syndrome. This review summarizes  
28          outcomes on metabolic syndrome biomarkers in recent clinical trials supplementing anthocyanins,  
29          catechins, beta-glucan, and n-3 long chain polyunsaturated fatty acids, focusing mainly on anti-  
30          obesity effects. Overall, it is clear that the level of evidence for the effectiveness varies not only  
31          among the different bioactives but also **among the different putative health benefits suggested for the**  
32          **same bioactive. Limited evidence may be** due to the low number of controlled intervention trials or  
33          to **inconsistencies in trial design i.e.** duration, dose and/or the way of bioactive supplementation  
34          (extracts, supplements, rich or enriched food). **At present, the question “are bioactives effective in**

35 weight management and prevention of metabolic syndrome?" remains inconclusive. Thus, a common  
36 effort to harmonize the study design of intervention trials focusing on the most promising bioactive  
37 molecules is urgently needed to strengthen the evidence of their potential in the treatment of obesity,  
38 metabolic syndrome and related diseases.

39

40 Key words: anthocyanins, beta-glucan, catechins, n-3 long chain polyunsaturated fatty acids,  
41 metabolic syndrome

42

43

#### 44 **Introduction**

45 A fundamental principle of nutrition and metabolism is that body weight (BW) change is associated  
46 with an imbalance between the energy **intake and energy expenditure**. On this basis, it is commonly  
47 **and simplistically** theorized that some people become overweight simply because they eat too much  
48 and exercise too little. Although this is theoretically true, different contributors to energy balance  
49 must be considered and need a better understanding. For example, diet composition, nutrient  
50 bioavailability and bioactives could have a role in energy balance.

51 The different thermic effects of macronutrients could result in different energy expenditure. **For**  
52 **example, higher protein diets have been shown to be** more conducive to weight loss than lower protein  
53 diets<sup>(1)</sup>. The Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial examined  
54 the role of macronutrients on overall energy expenditure and its components under well-controlled  
55 conditions<sup>(2)</sup>. This randomized trial involving 811 overweight adults evidenced that low energy,  
56 isocaloric diets with different macronutrient ratio (fats:proteins:carbohydrates = 20:15:65; 20:25:55;  
57 40:15:45 or 40:25:35) were equally successful in promoting weight loss and the maintenance of  
58 weight loss over a two-year period.

59 Low glycemic load (GL) diets have been reported to improve weight-loss maintenance<sup>(3)</sup>. This could  
60 be ascribed at least in part to a reduced nutrient availability due to the high fiber content of low GL  
61 diets.

62 Some bioactives have been shown to exert anti-obesity effects through suppression of appetite,  
63 inhibition of carbohydrate and lipid digestive enzymes<sup>(4)</sup>, regulation of lipid metabolism, and increase  
64 in energy expenditure<sup>(5)</sup>; and they have been considered as a new strategy for the development of anti-  
65 obesity functional foods.

66 Anthocyanins (ACN), catechins (C), beta-glucan (BG), and n-3 long chain polyunsaturated fatty acids  
67 (n-3 LCPUFA) are among the most promising candidates, although clinical trials using the pure  
68 bioactives or bioactive-rich foods demonstrate inconsistent findings. This review examines the main

recent findings coming from clinical intervention studies using the above cited bioactives . Few trials specifically address the effect of bioactives on BW or body mass index (BMI), but evidence regarding these parameters can come from trials focused on metabolic syndrome (MetS). Abdominal obesity is an important criterion for MetS diagnosis along with glucose intolerance, dyslipidemia, and hypertension<sup>(6)</sup>, and the selected bioactives have been used in several trials aimed to improve MetS. Summarized outcomes on MetS biomarkers in clinical trials supplementing ACN, C, BG and n-3 LC PUFA are outlined below, focusing on anti-obesity effects.

## Anthocyanins

**Anthocyanins (ACNs)** comprise a subgroup of flavonoids abundant in many fruits and vegetables, in particular, berries and grapes and their products such as juice and wine. Particularly rich in anthocyanins are berries such as **blackberries, black currants, black elderberries, and blueberries** with some varieties producing around 400-500mg ACN/100g<sup>(7, 8)</sup>. ACNs are water-soluble glycosylated pigments produced through plant secondary metabolism and responsible for the red, purple or blue colours. Most predominant ACN compounds are derived from pelargonidin, cyanidin, delphinidin, petunidin, peonidin and malvidin base structures, differing with regards to position and number of hydroxyl groups, degree of methylation, type and number of sugar moieties, ultimately leading to a large diversity of anthocyanins and their composition in different plants. The major ACN found in most plants is cyanidin-3-glucoside.

Reduction of weight gain following ACN supplementation in rodents has been associated with modulation of hepatic lipid metabolism, such as reduction of SREBP-1 mRNA levels, inhibition of enzymes involved in fatty acid and triacylglycerol (TG) synthesis and upregulation of lipolytic enzymes<sup>(9)</sup>. Furthermore, energy expenditure has been found accelerated in high-fat diets (HFD)-induced obese mice following blackberry and blueberry ACN supplementation<sup>(10)</sup>. Similarly, Solverson *et al.*<sup>(11)</sup> reported an increase in fat oxidation in a recent RCT in 27 overweight or obese males given blackberries (1500 mg/d) with high fat diets for seven days.

Daneshzad *et al.*<sup>(12)</sup> conducted a systematic review and meta-analysis of 19 RCTs evaluating effects of ACN supplementation on cardio-metabolic biomarkers including BW, BMI, waist circumference (WC), blood pressure (BP), lipid profile and glycaemic status. Duration of supplementation ranged from 1-96 weeks with ACN doses ranging from 31.5-1050 mg per day. While there was no significant effect of ACN supplementation on BW, WC, BMI, BP (systolic and diastolic), a sub-group analysis revealed that ACN intake for more than 12 weeks led to a 2.42 kg reduction in BW (MD: -2.42kg; 95% CI: -4.46, -0.38; P=0.020) and a 0.75 kg/m<sup>2</sup> decrease in BMI (MD: -0.75 kg/m<sup>2</sup>; 95% CI: -1.38, -0.23; P=0.005). Given the overall lack of effect on anthropometric markers and BP, duration as well as ACN dose may be the most likely sources for heterogeneity observed among different trials. This

103 is in line with Amiot *et al.*<sup>(13)</sup> who included six ACN supplementation studies in their systematic  
104 review on the effects of dietary polyphenols on MetS markers and reported highly variable results on  
105 BMI, WC, BP, lipid profile and glucose metabolism which are likely to relate to the different amounts  
106 of ACN provided through different berry food products (berry type, juice or powder product, extract)  
107 given over a supplementation periods of 6-8 weeks. Most effective was a mixture of berries (bilberry,  
108 blueberry, sea buckthorn) taken daily over 8 weeks to reduce BMI and WC<sup>(14)</sup>; aronia extract (300mg  
109 daily over 2 months) was able to significantly reduce BMI<sup>(15)</sup>. Conversely, a 6-week daily  
110 supplementation with freeze dried strawberry powder (equivalent to 500g fresh strawberries) caused  
111 no changes in anthropometric indices and serum glucose<sup>(16)</sup>.

112 ACN may exert hypoglycaemic effects through a combination of mechanisms including inhibition of  
113 carbohydrate digestion through inhibition of salivary and pancreatic  $\alpha$ -amylase and  $\alpha$ -glucosidase,  
114 inhibition of intestinal glucose absorption<sup>(17)</sup>, stimulation of insulin secretion<sup>(18)</sup> and increased glucose  
115 uptake in peripheral tissues through upregulated GLUT4 and its utilization<sup>(19, 20)</sup>. Furthermore,  
116 cyanidin-3-glucoside has been shown to lead to increased differentiation of pre-adipocytes into  
117 smaller and insulin-sensitive adipocytes<sup>(21)</sup> and exerts insulin like effects in human adipocytes by  
118 upregulating PPAR $\gamma$  activity<sup>(22)</sup>. Other mechanisms related to decreased insulin resistance (IR)  
119 involve activation of AMPK and IRS-1 and reduced inflammation<sup>(9)</sup>. In addition, anthocyanins may  
120 act in the gut to modulate postprandial blood glucose, insulin and incretin response<sup>(23)</sup>.

121 High intake of ACNs has been associated with significantly lower peripheral IR and hs-CRP levels<sup>(24)</sup>.  
122 Soltani *et al.*<sup>(25)</sup> has shown that the daily consumption of ACN-rich cornelian berry (*Cornus mas L.*)  
123 improved glycaemic control significantly by increasing insulin and reducing HbA1 levels in type 2  
124 diabetic patients; and a 12 week RCT in 138 Chinese adults with prediabetes or early untreated  
125 diabetes revealed that purified ACN favourably affects glycaemic control and lipid profile, in  
126 particular in patients with elevated metabolic markers<sup>(26)</sup>. As well, a recent systematic review and  
127 meta-analysis involving 32 RCTs with a minimum duration of 2 weeks, demonstrated a consistently  
128 improved glycaemic control (reduced fasting glucose, 2 h postprandial glucose and glycated  
129 haemoglobin) in both healthy and metabolically diseased populations, though in particular in subjects  
130 with existing hyperglycaemia<sup>(27)</sup>. This review also indicated significant reductions in total cholesterol  
131 and low density lipoprotein (LDL) levels across the 32 RCTs.

132 Daneshzad *et al.*<sup>(12)</sup> could not confirm effects on HbA1c, serum insulin and blood lipid profile in their  
133 systematic review/meta-analysis when all 19 studies were included. Sub-grouping for interventions  
134 over 300mg ACN/day and duration over 12 weeks significantly lowered HOMA-IR (-21%). ACN  
135 supplementation periods over 12 weeks significantly increased HDL-C and reduced LDL-C levels,  
136 and ACN supplementation >300 mg significantly reduced total cholesterol by 6.69 mg/dL and LDL-

137 C levels by 8.60 mg/dL. Hassellund *et al.*<sup>(28)</sup>, investigating the impact of ACN on cardiovascular risk  
138 factors and inflammation in pre-hypertensive men, emphasize the importance of ACN  
139 supplementation period over the dose in intervention studies, which is confirmed by Zhu *et al.*<sup>(29)</sup>  
140 demonstrating significantly reduced LDL-C and increased HDL levels after 24 weeks of ACN  
141 supplementation. Further, Alvarado *et al.*<sup>(30)</sup> confirmed that LDL-C only decreased significantly after  
142 12-weeks and not after 4 and 8 weeks of ACN-supplementation.

143 Also, in the systematic review/meta-analysis by Daneshzad *et al.*<sup>(12)</sup> significant reductions for total  
144 cholesterol, triglycerides and LDL-C, and significant increase for HDL were observed among patients  
145 with hypercholesterolaemia, indicating that ACN supplementation may provide a higher benefit to  
146 these patients in comparison to healthy individuals. Similar conclusions were drawn from a previous  
147 systematic review of Wallace *et al.*<sup>(31)</sup> evaluating effects of purified ACNs and ACN-rich extracts on  
148 markers of cardiovascular diseases (CVD) (total cholesterol, triglycerides, LDL-C, HDL-C, BP) in  
149 healthy and diseased subjects in supplementation trials ranging from 3-24 weeks and ACN doses  
150 from 7.4-640 mg/day stating that largest reductions (particularly LDL-C) could be achieved in  
151 subjects with elevated levels.

152 To summarize, ACN and ACN-rich foods are generally accepted to benefit (maintaining) healthy  
153 BW, improvement of glucose and lipid metabolism which has been demonstrated at least partially in  
154 a number of intervention studies. Variations seen in outcomes of individual studies may be due to  
155 varying ACN dose and duration of intervention trials with a duration of 12 weeks and amounts around  
156 300mg ACN be considered beneficial. However, the source of ACN *per se* might have a strong impact  
157 on its effectivity. **Highly methylated ACNs such as malvidin and petunidin have demonstrated to be**  
158 **more effective at reducing negative metabolic consequences (body composition, energy expenditure,**  
159 **mitochondrial dysfunction) in HF-diet fed C57BL/6 mice<sup>(32)</sup>.** At present, to classify ACNs and/or  
160 ACN-sources based on their effectiveness is not possible. Future studies need to consider the ACN  
161 concentration and profile, the possible synergism between different ACN and other bioactives within  
162 the same source, as well as factors such as processing and intake patterns.

### 163 **Catechins**

164 **Catechins (Cs)** are a group of polyphenols, flavan-3-ols, belonging to one of most common group of  
165 polyphenolics in the human diet, the flavonoids. The name catechin is derived from Cutch tree  
166 (*Acacia catechu L.f.*). Catechins are present in abundant concentrations in a variety of fruits,  
167 vegetables and plant-based beverages such as apple, berries, cacao beans, black soy bean, hops, tea,  
168 beer, wine and fruit juice<sup>(33)</sup>. The consumption of food rich in Cs is associated with potential health  
169 benefits partly based on the antioxidant properties of polyphenols<sup>(34)</sup>. The chemical structure of Cs  
170 consists of two benzene rings (A- and B-rings) and a dihydropyran heterocycle (the C-ring) with a

171 hydroxyl group on carbon 3. There are two chiral centers on the molecule on carbons 2 and 3.  
172 Catechin stereoisomers in cis ((-)-epicatechin) or trans ((+)-catechin) configuration, with respect to  
173 carbons 2 and 3, are flavan-3-ol compounds. Through esterification with gallate groups, flavanols can  
174 form gallic acid conjugates epicatechin ECG), epigallocatechin (EGC), and epigallocatechin gallate  
175 (EGCG). Condensed Cs are obtained via polymerization. The most common oligomers derived from  
176 epicatechin are A-type and B-type procyandins<sup>(35)</sup>.

177 This review of the clinical trials performed to evaluate the potential health effects of Cs on reducing  
178 the risk factors of MetS is focused on the results of human trials performed with food or food  
179 supplement or extracts rich in catechins. Studies have mainly been performed with cocoa and green  
180 tea, which are considered the richest dietary sources of Cs. Particularly, cocoa contains catechin,  
181 epicatechin and oligomers, and green tea is rich in EGCC, which is considered to be the most potent  
182 catechin and responsible for its health properties<sup>(36, 37)</sup>.

183 Hibi *et al.*<sup>(38)</sup> studied the effects of continual intake of green tea catechins (GTCs) in MetS. In  
184 particular, the authors led a post-hoc pooled analysis of data obtained from published reports (six  
185 human trials) to assess the effects of continual intake of GTC-containing beverages (540-588 mg/day)  
186 on abdominal fat area reduction and improvements in MetS (total 921 subjects). The studies were run  
187 in healthy Japanese adults (BW: 71,8±10 kg; WC: 88,9±7,3 cm; BMI: 26,8±2,3 kg/m<sup>2</sup>) that consumed  
188 GTCs for 12 weeks. Volunteers were categorized as Pre-MetS and MetS at the initiation of the trial.  
189 Results show that BW and BMI were significantly lower in the group receiving the high GTC dose,  
190 mean 564 ±19 mg GCT/day, (BW: -1.69 kg, 95%CI: -1.84 to -1.53; BMI: -0.65 kg/m<sup>2</sup>, 95%CI: -0.70  
191 to -0.59 from baseline). WC and abdominal fat area (total fat area, visceral fat area, and subcutaneous  
192 fat area) decreased significantly from baseline in the high GTC group, and the decrease was  
193 significantly greater than that in the low GTC group (35±50 mg/day) ( $P < 0.001$ ). Moreover, the  
194 analysis of the subclass exposed that in both groups, low (LC) and high (HC) catechins, an  
195 improvement was observed in the proportion of subjects who improved from Pre-MetS to healthy,  
196 and from MetS to healthy or Pre-MetS, in 30.2% of subjects in the LC group and 41.5% of subjects  
197 in the HC group. However, the rate was significantly higher in the high catechin group than in the LC  
198 group ( $P = 0.024$ , chi-square test).

199 In contrast, a randomized, doubled-blind, placebo-controlled study by Mielgo-Ayuso *et al.*<sup>(34)</sup>  
200 reported no effect after the consumption of 300mg EGCC mg/d for 12 weeks in 83 premenopausal  
201 women (BMI 30.0-39.9 kg/m<sup>2</sup>). It did neither improve BW nor metabolic risk factors such as blood  
202 lipids.

203 A review carried out by Keske *et al.*<sup>(39)</sup> showed the heterogeneity of the results in trials aimed to link  
204 consumption of EGCG/green tea with glucose tolerance and insulin sensitivity. In patients with type

205 2 diabetes, green tea extract (EGCG 860 mg/d) for 16 weeks significantly reduced HOMA-IR,  
206 glycosylated hemoglobin (HbA1c), and fasting insulin levels<sup>(40)</sup>, and consumption of more than 3  
207 cups of tea per day was associated with a 17-35% lower risk of type 2 diabetes<sup>(41)</sup>. Shimada *et al.*<sup>(42)</sup>  
208 revealed that oolong tea consumption for 4 weeks (45 mg/d of EGCG) significantly increases plasma  
209 adiponectin levels by 9.9% and lowers HbA1c levels by 3.3% in patients with various coronary risk  
210 factors. Additionally, there was a slight, but not significant, decrease in the fasting plasma glucose  
211 levels. Hosoda *et al.*<sup>(43)</sup> used a higher dose of oolong tea treatment (EGCG 390 mg.d<sup>-1</sup>) for 4 weeks  
212 and reported lower fasting plasma glucose levels in people with type 2 diabetes. In contrast, green tea  
213 consumption (540 mg/d polyphenols, EGCG content unknown) for 2 months had no apparent effect  
214 on metabolic markers such as fasting serum glucose and insulin, HbA1c, and HOMA-IR<sup>(44)</sup>. The  
215 proportion of flavanols (ratio of catechins is different in oolong tea than in green tea) and the study  
216 duration are critical aspects to modulate glucose metabolism positively.

217 The results of intervention studies indicate that consumption of flavan 3-ols is associated with an  
218 improvement of lipid homeostasis parameters such as HDL-C and LDL-C. Tokede *et al.*<sup>(37)</sup> analyzed  
219 10 RCTs of interventions (total 320 participants) administering dark chocolate/cocoa products for 2  
220 to 12 weeks. Eight of the studies were comparing flavanol-rich cocoa or dark chocolate with either  
221 flavanol-poor white chocolate or a matching placebo. One study compared milk chocolate with cocoa  
222 butter and one compared a supplemented diet with dark chocolate and cocoa powder with an  
223 unsupplemented diet. Therefore, the intake of catechins was heterogeneous, from 963 mg/day to 88  
224 mg/day compared with control intake from 0-75 mg catechins. The differences in catechin intake  
225 between cocoa/chocolate group and control ranged from 8.74% to more than 100%. The authors  
226 reported a significant reduction in serum LDL-C and total cholesterol levels (-5.90 mg/dl and -6.23  
227 mg/dl, respectively) (data as mean difference of the results of the 10 studies). No statistically  
228 significant effects were observed for HDL-C and triglyceride (TG). Hooper *et al.*<sup>(45)</sup> described the  
229 marginally significant effects of cocoa products on LDL-C (-0.07 mmol/L; 95% CI: -0.13, 0.00  
230 mmol/L) and HDL-C (0.03 mmol/L; 95% CI: 0.00, 0.06 mmol/L) cholesterol (data referred as mean  
231 difference of the differences in each study between cocoa group and control).

232 Hartley *et al.*<sup>(46)</sup> carried out an analysis of RCTs lasting at least 3 months which investigated the  
233 effects of black or green tea or tea extracts involving healthy adults or those at high risk of CVD. The  
234 global analysis of the consumption of black tea (1 g extract/day, 1.29 g black tea polyphenols/day;  
235 three serving of black tea (200 mL/serving) and 318 mg black tea catechins/day) was found to produce  
236 statistically significant reductions in LDL -C (mean difference -0.43 mmol/L, 95% CI -0.56 to -0.31).  
237 Green tea (58.91 mg catechin in green tea, 500 mg green tea polyphenols/day, 375 mg green tea  
238 extract/day, 200 mg theanine and 400 mg decaffeinated catechin green tea extract/day) was also found

239 to produce statistically significant reductions in total cholesterol (mean difference MD -0.62 mmol/L,  
240 95% CI -0.77 to -0.46) and LDL-C (MD -0.64 mmol/L, 95% CI -0.77 to -0.52). When both tea types  
241 were analyzed together they showed favorable effects on LDL-C (MD - 0.48 mmol/L, 95% CI -0.61  
242 to -0.35).

243 The meta-analysis of Desch *et al.*<sup>(47)</sup> and Hooper *et al.*<sup>(45)</sup> confirmed the blood pressure-lowering  
244 capacity of flavanol-rich cocoa products. Desch *et al.*<sup>(47)</sup> analyzed 297 participants including six  
245 cross-over and four parallel-group designs. **Although the studies displayed a diverse spectrum of**  
246 **treatment regimens (duration from 2 to 8 weeks and intake (from 6.8 mg/d- 902 mg/d flavanol),**  
247 results revealed that the mean blood pressure reduction was -4.5mmHg (95% CI -5.9 to -3.2; I<sup>2</sup>=89%)  
248 for systolic BP and -2.5mmHg (95% CI -3.9 to -1.2; I<sup>2</sup>=90%) for diastolic BP. Hooper *et al.*<sup>(45)</sup>  
249 reviewed the effects of chocolate, cocoa, and flavan-3-ols including 42 acute or short-term chronic  
250 ( $\leq$ 18 wk) RCTs that comprised 1297 participants. They observed reductions in diastolic BP (-1.60  
251 mm Hg; 95% CI: -2.77, -0.43 mm Hg) and mean arterial pressure (-1.64 mm Hg; 95% CI: -3.27, -  
252 0.01 mm Hg). **Although some studied did not identify dose-dependent effects of ECG, subgrouping**  
253 **by ECG dose suggested greater effects for systolic and diastolic BP at doses >50 mg/d.** In the above  
254 reported meta-analyses by Hibi *et al.*<sup>(38)</sup> a significant decrease of systolic BP compared to baseline  
255 was observed only in the high catechin group (-1.1mmHg, 95%CI: -2.1 to -0.1), (P < 0.01). The 11  
256 RCTs analysed by Hartley *et al.*<sup>(46)</sup> evidenced that black tea consumption significantly **reduced**  
257 systolic BP (MD -1.85 mmHg, 95%CI -3.21 to -0.48), and green tea consumption significantly  
258 decreased both systolic and diastolic BP (MD -3.18 mmHg, 95% CI -5.25 to -1.11 and MD -3.42,  
259 95% CI -4.54 to -2.30, respectively).

260 Most of the studies in the literature have been performed using Cs from tea or cocoa, and have  
261 evidenced that the dose exerting positive effects strongly depends on the physiological parameters  
262 that are being studied. Overall, Cs from tea seem to be effective in most of the MetS risk factors at a  
263 daily intake above 390 mg. The effect of cocoa's Cs is evident on BP with an intake from 6.5 mg/day.  
264 Overall, several *in vitro* and *in vivo* animal studies are elucidating the potential mechanisms of action  
265 of Cs and they **are on the way to demonstrate that** flavan 3-ols can modulate metabolic pathways of  
266 the glucose and lipid metabolism and blood pressure. It has been reported that EGCG up-regulates  
267 LDLr mRNA, reduces ApoB levels and inhibits pancreatic lipase, thereby reducing the absorption of  
268 dietary lipids<sup>(48)</sup>. Therefore, the modulation of molecules in lipid and glucose metabolism and the  
269 reduction on the delivery of proinflammatory cytokines as IL-6<sup>(49)</sup> by catechins could contribute to  
270 reducing cholesterolemia (LDL and total cholesterol) and BW. *In vitro* studies in several cell types  
271 (myocytes, adipocytes and hepatocytes) have reported that green tea or EGCG have insulin-mimetic  
272 metabolic actions. EGCG stimulates the uptake of glucose by stimulation of GLUT4 translocation<sup>(39)</sup>.

273 Analysis in endothelial cells show the enhancement of nitric oxide production by EGCG<sup>(48, 50)</sup>. Apart  
274 from the metabolic regulation, recent studies are focusing on assessing the epigenetic modulation of  
275 candidate genes of MetS by flavan 3-ols<sup>(48)</sup>.

276 Although these mechanisms could justify positive effects of Cs in humans, results of clinical  
277 intervention studies are still controversial. This is probably due to discrepancies among studies,  
278 including varying experimental designs, type and doses of Cs. Further research is needed to draw  
279 robust conclusions.

280 **Beta-glucan**

281 **Beta-glucan (BG)** is a non-starch polysaccharide found in the cell walls of endosperm and aleurone  
282 cells of grains. BG consists of short  $\beta$ -(1,4)-D-glycans (cellotriosyl and cellotetraosyl units) linked to  
283 each other by  $\beta$ -(1,3) linkages leading to polymers of high molecular weight ranging from 8-200  
284 kDa<sup>(51)</sup>. This specific chemical structure is responsible for its physical properties, such as high  
285 solubility and viscosity which may contribute to the health benefits attributed to BG<sup>(52)</sup>, in particular  
286 those attributed to improvements of cardiometabolic health. Oat and barley are rich in BG, and most  
287 of the studies have been performed using BG from oat or barley.

288 Elevated WC is one of the criteria for MetS. However, clinical studies on BG have not focused on  
289 this anthropological parameter. A 4% decrease of the WC was observed following adoption of a  
290 healthy diet that included ‘viscous fibres’ amongst other dietary improvements<sup>(53)</sup>, which also saw  
291 improvement in a number of metabolic markers including fasting glucose, total and HDL-C. Beck  
292 et al.<sup>(54)</sup> observed a significant effect of oat BG consumption (5-9 g/day) at breakfast on BW and  
293 WC, together with improvements in metabolic markers and alterations in levels of satiety hormones  
294 including leptin, and peptide YY (PYY). The study, however, showed that an energy restricted diet  
295 had similar effects compared to oat BG consumption, which did not enhance the effectiveness of  
296 energy restriction. It is worth noting that the EFSA panel did not find sufficient evidence to  
297 substantiate a link between BG consumption and a reduction in appetite or **BW** (maintenance or  
298 achievement of normal BW)<sup>(55)</sup>, although the panel did not consider evidence related to waist  
299 circumference.

300 Conversely, EFSA supported a health claim stating that regular consumption of BG contributes to the  
301 maintenance of normal blood cholesterol concentrations for foods that provide “at least 3 g/d of BG  
302 from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed  
303 BGs in one or more servings”<sup>(55)</sup>. The US Food and Drugs Administration (FDA) provided a similar  
304 recommendation<sup>(56)</sup>.

305 A meta-analysis of epidemiological studies reported beneficial effects on blood lipids associated with  
306 consumption of soluble fibre from both oats and barley, but reported high levels of heterogeneity and

307 called for well controlled intervention studies<sup>(57)</sup>. A meta-analysis of randomised controlled trials  
308 showed that oat BG at doses higher than 3 g/day reduced LDL-C and total cholesterol significantly  
309 compared to control, with little or no effect on HDL-C and TG irrespective of dose or study  
310 duration<sup>(58)</sup>. The authors specified that the effectiveness of oat BG is linked to its high molecular  
311 weight and associated physicochemical properties, however called for more dose response and longer  
312 studies to evaluate impacts of chronic consumption of oat BG in healthy and MetS populations.  
313 Ibrugger *et al.*<sup>(59)</sup> compared the effects of BG from oats and barley and showed that neither affected  
314 blood lipids significantly compared to the control. However, the consumption of 3.3 g/day oat BG  
315 led to the largest observed decrease in total cholesterol and LDL-C, as well as significantly reducing  
316 TG. The authors identified a lack of systematic studies, with great differences amongst studies in  
317 terms of study foods, dose and study duration. Few of the intervention studies investigated the dose-  
318 effect relationship between oat BG and blood cholesterol. Biorklund *et al.*<sup>(60)</sup> reported that consuming  
319 a drink containing 5g/day oat BG resulted in a 6.7% decrease in LDL-C, while consumption of the  
320 drink containing 10g/day oat BG reduced LDL-C by only 3.7%, compared to control drink.  
321 Kerckhoffs *et al.*<sup>(61)</sup> highlighted that processing of oats could have an adverse effect on the cholesterol  
322 lowering effect. Charlton *et al.*<sup>(62)</sup> showed that 1.5 g/day provided as cereal flakes was just as effective  
323 as 3 g/day provided as porridge in lowering blood cholesterol. Wolever *et al.*<sup>(63)</sup> showed the  
324 importance of molecular weight for the effectiveness of oat BG towards cholesterol markers<sup>(63)</sup>. The  
325 impact of processing on oat BG properties has been recently reviewed by Grundy *et al.*<sup>(64)</sup>. In healthy  
326 people, BG consumption does not appear to affect lipid homeostasis<sup>(65)</sup>.  
327 Epidemiological studies have supported the association between whole grain intake and improved  
328 metabolic risk factors for type 2 diabetes and metabolic syndrome<sup>(66, 67)</sup>. The fasting glucose  
329 concentrations decreased across increasing quartile categories of whole-grain intake. However, few  
330 clinical trials have focused on the impact of the consumption of BG on glucose metabolism. Many  
331 studies investigating BG and lipid homeostasis have also investigated impacts on glucose  
332 homeostasis. The EFSA panel supported a claim that consuming 4g of BG from oats or barley for  
333 each 30g of available carbohydrate decreased post-prandial **glycaemic** response without  
334 disproportionately increasing **insulin** response. The effect was observed when BG was incorporated  
335 into carbohydrate-rich food (e.g. bread or pasta) and when combined into a meal<sup>(55)</sup>. Consuming at  
336 least 4g BG per meal, from either oats or barley, and where the BG is soluble and has a MW  
337 >250 000 g/mol is sufficient to significantly reduce post-prandial area under curve (AUC) by  
338 27±3 mmol·min/l for meals with ~30–80g of available carbohydrates<sup>(68)</sup>. He *et al.*<sup>(69)</sup> carried out  
339 a meta-analysis of controlled intervention trials, and showed that consumption of either wholegrain  
340 oats or BG extracted from oats **was** associated with strong significant reducing effects on fasting

341 glucose and fasting insulin in type 2 diabetics, but no effect on hyperlipidemic subjects. A  
342 moderate effect was observed for obese subjects without hyperlipidemia. A long-term (six months)  
343 substitution of regular white bread with a functional bread enriched with fibre (7.62 g/100g of bread,  
344 mostly BG) in the everyday diet of subjects with type 2 diabetes induced no statistical difference on  
345 the fasting glucose level, but a significantly decrease was observed for the post-prandial plasma  
346 glucose ( $P = 0.001$ ) and mean plasma glucose ( $P = 0.02$ ) with the 'functional bread' compared to the  
347 control bread<sup>(70)</sup>. In this study, other metabolic markers such as blood lipids, blood pressure were not  
348 affected.

349 Few clinical trials have specifically studied the effects of the consumption of BG on blood pressure.  
350 The results of the different studies show discrepancies. Past results obtained with healthy volunteers  
351 generally did not demonstrate an effect of the consumption of fibres on blood pressure compared to  
352 low-fibre grain supplementation<sup>(71)</sup>. However, a recent meta-analysis concluded that systolic and  
353 diastolic BP could be reduced by 2.9 mmHg (95% CI 0.9 to 4.9 mmHg) and 1.5 mmHg (95% CI 0.2  
354 to 2.7 mmHg) respectively by diets rich in BG, for a median difference in BG of 4 g in healthy  
355 volunteers<sup>(72)</sup>. The consumption of BG should thus help to manage BP of non-healthy people,  
356 especially people at risk of MetS. In 2006, Behall *et al.*<sup>(73)</sup> demonstrated the effects of consuming  
357 controlled portions of whole-grain rice and barley BG on BP in 25 overweight/obese mildly  
358 hypercholesterolemic women. Both wholegrain rice and barley BG interventions led to significant  
359 decreases in diastolic BP and the mean arterial pressure, especially in post-menopausal women. In a  
360 randomized cross-over design, the consumption of a diet enriched in legumes and barley by  
361 overweight women for 4 weeks induced a significantly reduction (-3 %,  $P < 0.05$ ) of the diastolic BP  
362 but no effect was observed on systolic BP compared to the equivalent diet without legumes and  
363 barley<sup>(74)</sup>. A similar observation was made in healthy and obese men and women consuming  
364 multifunctional diets that included BG amongst other health enhancing constituents<sup>(53)</sup>. However, it  
365 is difficult to dissociate the effect of BG from other constituents in the diet.

366 Summarizing, there is strong and consistent evidence that consumption of BG impacts on lipid  
367 metabolism, with strong caveats relating to the dose and molecular size required for effects. There  
368 are multiple mechanisms associated with the effects of BG on lipid metabolism which may be acting  
369 in concert to exert positive effects. Proposed mechanisms include increased gut permeability<sup>(75)</sup>,  
370 reduced lipid digestion and absorption<sup>(52)</sup>, decreased bile reabsorption through physical barrier and  
371 bile colonic metabolism<sup>(76)</sup>, increased bile acid production and short chain fatty acid metabolism<sup>(77)</sup>  
372 which impact on cholesterol homeostasis. **There is also strong evidence supporting a role for BG**  
373 **in control of post-prandial glucose, but its effect may be attributed to fibre in general, rather than**  
374 **specifically to BG.**

375 The evidence for other markers of MetS including BW, fasting glucose and BP are less well  
376 established. It is clear that further research is needed, also focusing on BG-matrix interactions and  
377 implications of food processing.

378 **n-3 long chain polyunsaturated fatty acids**

379 **n-3 long chain polyunsaturated fatty acids (n-3 LCPUFAs), namely eicosapentaenoic acid (EPA) and**  
380 **docosahexaenoic acid (DHA)** have been suggested as potential anti-obesity bioactives<sup>(5)</sup>, and growing  
381 evidence is emerging about the role of white adipose tissue (WAT) in mediating the beneficial effects  
382 of marine n-3 PUFAs in obesity-associated metabolic disorders. EPA and DHA have been shown to  
383 reduce BW and fat deposition in human clinical studies<sup>(78)</sup>. Their mechanism of action is supposed to  
384 be multiple. After consumption, these fatty acids are incorporated into cell membranes where they  
385 modulate membrane protein function, cellular signaling, and gene expression<sup>(79)</sup>. Incorporation of  
386 EPA and DHA into tissues may modify inflammatory and immune reactions, mainly by inhibiting  
387 pro-inflammatory interleukins, therefore counteracting low-grade chronic inflammation caused by  
388 obesity. It has been suggested that MetS is the consequence of adipose tissue abnormalities.  
389 Therefore, n-3 LCPUFA could target adipose tissue inflammation and improve systemic  
390 metabolism<sup>(80)</sup>. In addition, several trials indicate that n-3 LCPUFA reduce hypertension, total  
391 cholesterol (TC), and TG levels in the body, being a perfect candidate to develop nutritional strategies  
392 to counteract MetS.

393 In 2012 and 2013, the effects of EPA and DHA have been in the focus of two reviews emphasizing  
394 several limitations, including varying experimental designs, type and doses of n-3 PUFAs, making it  
395 impossible to draw robust conclusions<sup>(81, 82)</sup>. Other trials have been performed in the following years  
396 using supplements, fish oil or enriched foods.

397 In the single-blind, parallel trial described by Oh *et al.*<sup>(83)</sup> a placebo or n-3 PUFA **as supplement** (1,  
398 2, or 4 Omacor® capsules each containing 460mg EPA ethyl ester and 380mg DHA ethyl ester) were  
399 randomly administered to 176 patients with primary hypertriglyceridemia ( $> 150$  mg/dl) once daily  
400 for two months. n-3 PUFA treatment dose-dependently and significantly decreased TG and TG/HDL-  
401 C and improved flow-mediated dilation **but caused no significant modification in BMI compared with**  
402 **placebo**.

403 Likewise, no modification in fat-free mass, upper-body subcutaneous fat mass, and visceral fat mass  
404 across the intervention or between groups was observed in the prospective, randomized, placebo-  
405 controlled, double-blind study by Hames *et al.*<sup>(84)</sup> involving insulin-resistant, overweight or obese  
406 adults aged 18–65 y. Participants were randomly assigned to placebo (4.2 g oleic acid/day) or received  
407 a supplement containing 3.9g EPA+DHA/d. Although EPA and DHA concentration in plasma and  
408 adipose tissue significantly increased in the n-3 group, there was no improvement in adipose tissue

409 markers of inflammation. BMI (+0.7;  $P = 0.03$ ), percentage of body fat (0.9%;  $P = 0.009$ ), and leg  
410 fat mass (0.5 kg;  $P = 0.02$ ) increased for participants in both groups at the end of the intervention,  
411 and the changes were not different between groups.

412 Supplementation with n-3 LCPUFA did not improve the effect of a hypocaloric diet in the  
413 randomized, controlled trial by Tardivo *et al.*<sup>(85)</sup>. The trial included 87 postmenopausal Brazilian  
414 women with MetS, who were randomized to diet alone or diet plus omega-3 supplementation, 900  
415 mg/day. After 6 months, despite significant reductions in BMI and WC observed in both groups, there  
416 were no changes in body fat or muscle mass. Intervention with n-3 LCPUFA was associated with  
417 significant reduction in systolic (< 12.2%) and diastolic (< 8.2%) BP, serum TG concentration (<  
418 21.4%), and IR (< 13.1%) ( $P < 0.05$ ), as well as a reduction in serum IL-6 concentration (< 28.5%)  
419 ( $P = 0.034$ ).

420 In contrast, a significant effect on body fat upon n-3 LCPUFA supplementation was observed by  
421 Barbosa *et al.*<sup>(86)</sup>. In this double-blind, placebo-controlled, randomized clinical trial a supplement  
422 containing n-3 LCPUFA (3 g/d; 37% EPA and 23% DHA) or placebo (3 g/d sunflower oil) were  
423 administered for 2 months. Study participants were 80 men and women, aged 30 to 74 y, with some  
424 classic CVD risk factors (overweight, hypertension, dyslipidemia, diabetes, smoking) with or without  
425 treatment and without previous cardiovascular event. The n-3 group showed a significant reduction  
426 of body fat compared with the placebo group, without any significant modification in BW, BMI, and  
427 WC. In the treated group, an increase in serum adiponectin was detected. Adiponectin synthesis is  
428 inversely proportional to the amount of adipose tissue<sup>(86)</sup>; in animals, increased n-3 LCPUFA  
429 consumption is associated with increased adiponectin levels, however the results are controversial in  
430 humans<sup>(87)</sup>. Results of this trial confirm that n-3 LCPUFA consumption reduces body fat, leading to  
431 increased concentration of adiponectin and this, in turn, could further influence the reduction of fat  
432 mass.

433 Overall, although n-3 LCPUFA as supplements modify some MetS and CVD-related parameters they  
434 seem to have no effect on BW and BMI. On the contrary, a significant reduction of body fat could be  
435 related to the administration of supplements containing 3g/d EPA+DHA.

436 The effect of an increased dietary intake of n-3 LCPUFA could be different. The randomized  
437 controlled trial of the LIPGENE study<sup>(88)</sup> involved volunteers aged 35–70 y with a BMI of 20–40  
438 kg/m<sup>2</sup>, characterized by at least 3 of the following 5 criteria: high WC, high fasting glycemia, high  
439 TG, high BP, low HDL-C. Each subject was randomly stratified to one of 4 dietary interventions for  
440 12 weeks: high saturated fatty acids (HSFA); high monounsaturated fatty acids (HMUFA); n-3 diet  
441 including 1.24 g/d long-chain n-3 LCPUFAs with a ratio of 1.4 EPA:DHA; control diet, including  
442 control high-oleic acid sunflower seed oil capsules. Volunteers were stratified according to their IR.

443 MetS subjects without IR (lower HOMA-IR) showed improvement in metabolic risk factors related  
444 to MetS, such as obesity, blood pressure, and lipid markers, after consumption of the n-3 LCPUFA  
445 diet. In addition, in subjects without IR, WC was reduced after consumption of the control and n-3  
446 LCPUFA diets compared with the HSFA and HMUFA diets (all  $P < 0.05$ ).

447 Based on the evidence of the health benefits related to the consumption of oily fish<sup>(89)</sup>, some trials  
448 administered n-3LC PUFA as fish oil (FO), enriched oils or enriched food. The intervention study by  
449 Venturini *et al.*<sup>(90)</sup> included 102 patients (81 women and 21 men) with MetS (mean age  
450  $51.45 \pm 8.27$  y) aimed to compare extra virgin olive oil (OO) and FO effects, also investigating their  
451 possible synergism. Patients in the control group (CG) were instructed to maintain their usual diet;  
452 FO group received 3 g/d of FO (10 capsules, each one containing 180mg EPA and 120mg DHA); OO  
453 group received 10 mL/d of OO; and the fourth group (FOO) received 3 g/d of FO and 10 mL/d OO.  
454 After 90-d intervention, no intragroup changes in anthropometric parameters were observed  
455 compared to baseline. In the FOO group, after treatment a significant decrease in LDL-C, and  
456 TC/HDL-C and LDL-C/HDL-C indexes was observed compared with baseline.

457 Fifty-nine subjects with early-stage T2D or MetS participated in an 8-week, randomized, single-blind,  
458 parallel intervention study<sup>(91)</sup>. Individuals received either corn oil (CO), a botanical oil (BO)  
459 combination (borage [*Borago officinalis* L.]/echium oil [*Echium plantagineum* L.]) or FO (EPA 3.58  
460 g/d and DHA 2.44 g/d). FO supplementation induced a marked increase in serum levels of n-3  
461 LCPUFAs, HDL-C and insulin, and a decrease in serum TG. No indication of the effect on  
462 anthropometric data were reported by the researchers.

463 A randomized, cross-over, 5 diet period, controlled feeding study was conducted by Liu *et al.*<sup>(92)</sup> on  
464 130 participants with BMI between 22 to 40 kg/m<sup>2</sup> with central obesity plus at least one other MetS  
465 criteria. Five treatment oils: Canola oil, CanolaOleic (high-oleic acid canola oil), CanolaDHA (high-  
466 oleic acid canola oil with DHA), Corn/Saff (Corn/Safflower oil), and Flax/Saff (Flax/Safflower oil)  
467 were incorporated into smoothies that participants consumed twice daily. The quantity of oil was  
468 calculated based on participant energy needs, and it provided 18% of total energy. The impact of each  
469 test diet on BW and body composition was low, and mainly on android fat mass that significantly  
470 decreased from baseline on the Canola and CanolaOleic oil diets only. The reduction in android fat  
471 mass was positively correlated with decreases in cardiometabolic risk factors including TG, systolic  
472 and diastolic BP after all diets except the Corn/Saff oil group.

473 In a double-blind randomized trial, 36 patients with MetS received 500 mL/day of semi-skimmed  
474 milk (placebo) or 500 mL/day of skimmed milk enriched with 275mg of EPA+DHA and 7.5g of  
475 oleate and underwent 24 weeks of high-intensity interval training<sup>(93)</sup>. Treatment did not increase n-3  
476 LC PUFA plasma concentration, and a similar decrease in BW, WC, body fat mass, trunk fat mass

477 and BP were observed in placebo and treated group. However, insulin sensitivity, serum  
478 concentration of C-reactive protein, and HDL-C improved only in the treated group.

479 As for supplements, the increase of n-3 LCPUFA intake by FO or enriched-food significantly  
480 improves different physiological parameters without clear effect on BW, BMI and other  
481 anthropometric parameters.

482 The effect of n-3 LCPUFA was also investigated in combination with other bioactives. 78 individuals  
483 (33 men and 45 women), aged 35–70 years, with a large BMI (27–35 kg/m<sup>2</sup>) and WC (men >102 cm,  
484 women >88 cm) and at least one more component of the MetS were recruited in the trial reported by  
485 Bondia-Pons *et al.*<sup>(94)</sup>. Participants were randomly assigned to one of four different nutritional  
486 interventions for the duration of 8 weeks. Diets only differed for the content of n-3 LCPUFAs and  
487 polyphenols. Dependency network analysis showed a different pattern of associations between  
488 lipidomics, dietary, and clinical variables after the dietary interventions, but no modification in BMI  
489 or WC were observed in any group.

490 Foods with a combination of high-oleic acid canola oil-DHA (HOCO-DHA) and barley BG have  
491 been used in the CONFIDENCE trial, a randomized, single-blind crossover trial with four treatment  
492 phases of 28 days each<sup>(95)</sup>. The possible synergism between DHA and other bioactives was also in  
493 the focus of the EU project PATHWAY-27 (Pivotal assessment of the effects of bioactives on health  
494 and wellbeing. From human genome to food industry)<sup>(96)</sup> that investigated the role and mechanisms  
495 of action of DHA, oat BG, and AC, alone and in combination, in the counteraction of MetS  
496 considering them not as stand-alone molecules but as ingredients of food. In PATHWAY-27, three  
497 monocentric, parallel-arm, randomized, double blind pilot trials and a multicentre, randomized,  
498 placebo-controlled, parallel-arm dietary intervention study were performed on subjects at risk of  
499 MetS. At present, neither CONFIDENCE nor PATHWAY-27 results are available in the literature to  
500 report on the outcomes of potential combined effects in interventions involving DHA, BG (and AC).  
501 Based on available results, the increased intake of n-3 LCPUFA seem to have an effect on BW and  
502 BMI only if it is associated to modification of the whole diet so we can argue that it is not simply due  
503 to LCPUFA themselves. The effectiveness of n-3 LCPUFA on other parameters has been evidenced  
504 in trials using both supplements and enriched food with differences related to the daily dose, the  
505 duration of the intervention, and the EPA:DHA ratio.

## 506 Conclusion

507 Bioactives are a promising field of study for alternative strategies to reduce the onset and progression  
508 of MetS and its related pathologies including obesity. Some bioactives, such as ACs, Cs, BGs and  
509 n-3-LCPUFA, are considered good candidates since they have demonstrated positive effects in  
510 reducing MetS risk acting through different mechanisms. There are therefore opportunities to

511 investigate synergistic effects. However, there are still gaps in the evidence for some bioactives due  
512 to the low number of controlled intervention trials available or to inconsistent results among different  
513 trials likely caused by differences between dose and treatment time as well as the characteristics of  
514 the enrolled population. The inconsistencies could be also related to the source of the bioactive  
515 (extracts, supplements, enriched food, diet) that could impact on the bioavailability of the bioactive  
516 compounds. Bioavailability is seldom considered in intervention trials, neither its possible  
517 modification due to food processing. In addition, lifestyle factors, including dietary habits, play a  
518 fundamental role in intervention studies using bioactives.

519 Since bioactives are food components, their intake can be increased in different ways i.e. modifying  
520 the dietary pattern, including enriched foods in the diet (with or without modification of the dietary  
521 pattern) or administering supplements. Although the differences among these possible treatments are  
522 huge and evident, thus far no studies have been performed to compare the efficacy of diet vs enriched-  
523 foods vs supplements as bioactive vehicle. Anyway, conclusions from such trials could be difficult  
524 to interpret since bioactive consumption by dietary modification impacts on the dietary pattern. As  
525 an example, an increased n-3 LC PUFA consumption can only be achieved by including additional  
526 servings of oily fish, which is hard to achieve without reducing consumption of other food, while an  
527 increased C intake could be effected more simply through additional consumption of tea, with no or  
528 limited effect on the consumption of other food items. Also limiting the comparison to a specific  
529 bioactive, it is hard to extrapolate from different trials whether diet, enriched foods or supplements  
530 have acted more efficiently in exerting the claimed health effects mainly because the results of  
531 different studies are strongly dependent on the dosage, period of intervention, characteristics of the  
532 population, and the condition studied.

533 Dietary intervention trials aimed to verify the effectiveness of bioactive are more intriguing than drug  
534 trials. The effect of food bioactives is generally weaker than drugs, so it can be more easily masked  
535 by interfering factors. Apart from supplements, increased bioactive intake modifies the usual diet  
536 making difficult to discriminate the contribution of the dietary modification to the final effect. In  
537 summary, the demonstration of bioactive effectiveness is an uphill struggle. Nevertheless, it is worth  
538 tackling it since bioactives generally well accepted by consumers, generally safe and may be an  
539 alternative or additional therapeutic resource with considerable potential in the treatment of MetS.  
540 Therefore, increased effort should be made within the scientific community to design high quality  
541 clinical intervention trials with clearly defined and comparable supplementations and cohorts to  
542 increase the evidence for bioactive supplementation for the field to move forward towards evidence-  
543 based recommendations for prevention and targeted intervention strategies. Harmonization of study  
544 design for bioactive effectiveness would be a positive step towards gathering robust evidence. The

545 PATHWAY-27 consortium published scientific guidelines to guide the scientific community to  
546 design trials for bioactive effectiveness<sup>(97)</sup>.

547

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550

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#### 555 **Conflict of interest**

556 The Authors declare no conflict of interest.

557

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