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The role of bioactives in energy metabolism and metabolic syndrome

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Short title: Bioactives in the management of MetS

Abstract

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

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

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

Introduction

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

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

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

Some bioactives have been shown to exert anti-obesity effects through suppression of appetite, inhibition of carbohydrate and lipid digestive enzymes[4], regulation of lipid metabolism, and increase in energy expenditure[5]; and they have been considered as a new strategy for the development of anti-obesity functional foods.

Anthocyanins (ACN), catechins (C), beta-glucan (BG), and n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) are among the most promising candidates, although clinical trials using the pure 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[6], 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[7, 8]. 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[9]. Furthermore, energy expenditure has been found accelerated in high-fat diets (HFD)-induced obese mice following blackberry and blueberry ACN supplementation[10]. Similarly, Solverson *et al.*[11] 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.*[12] 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^2 decrease in BMI (MD: -0.75 kg/m^2 ; 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

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

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

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

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

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

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

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

Catechins

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

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

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

Hibi *et al.*[38] studied the effects of continual intake of green tea catechins (GTCs) in MetS. In particular, the authors led a post-hoc pooled analysis of data obtained from published reports (six human trials) to assess the effects of continual intake of GTC-containing beverages (540-588 mg/day) on abdominal fat area reduction and improvements in MetS (total 921 subjects). The studies were run in healthy Japanese adults (BW: 71,8±10 kg; WC: 88,9±7,3 cm; BMI: 26,8±2.3 kg/m^2) that consumed GTCs for 12 weeks. Volunteers were categorized as Pre-MetS and MetS at the initiation of the trial. Results show that BW and BMI were significantly lower in the group receiving the high GTC dose, mean 564 ±19 mg GCT/day, (BW: -1.69 kg, 95%CI: -1.84 to -1.53; BMI: -0.65 kg/m^2, 95%CI: -0.70 to -0.59 from baseline). WC and abdominal fat area (total fat area, visceral fat area, and subcutaneous fat area) decreased significantly from baseline in the high GTC group, and the decrease was significantly greater than that in the low GTC group (35±50 mg/day) ($P < 0.001$). Moreover, the analysis of the subclass exposed that in both groups, low (LC) and high (HC) catechins, an improvement was observed in the proportion of subjects who improved from Pre-MetS to healthy, and from MetS to healthy or Pre-MetS, in 30.2% of subjects in the LC group and 41.5% of subjects in the HC group. However, the rate was significantly higher in the high catechin group than in the LC group ($P = 0.024$, chi-square test).

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

A review carried out by Keske *et al.*[39] showed the heterogenicity of the results in trials aimed to link consumption of EGCG/green tea with glucose tolerance and insulin sensitivity. In patients with type

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

The results of intervention studies indicate that consumption of flavan 3-ols is associated with an improvement of lipid homeostasis parameters such as HDL-C and LDL-C. Tokede *et al.*[37] analyzed 10 RCTs of interventions (total 320 participants) administering dark chocolate/cocoa products for 2 to 12 weeks. Eight of the studies were comparing flavanol-rich cocoa or dark chocolate with either flavanol-poor white chocolate or a matching placebo. One study compared milk chocolate with cocoa butter and one compared a supplemented diet with dark chocolate and cocoa podwer with an unsupplemented diet. Therefore, the intake of catechins was heterogenous, from 963 mg/day to 88 mg/day compared with control intake from 0-75 mg catechins. The differences in catechin intake between cocoa/chocolate group and control ranged from 8,74% to more than 100%. The authors reported a significant reduction in serum LDL-C and total cholesterol levels (-5,90 mg/dl and -6,23 mg/dl, respectively) (data as mean difference of the results of the 10 studies). No statistically significant effects were observed for HDL-C and triglyceride (TG). Hooper *et al.*[45] described the marginally significant effects of cocoa products on LDL-C (-0.07 mmol/L; 95% CI: -0.13, 0.00 mmol/L) and HDL-C (0.03 mmol/L; 95% CI: 0.00, 0.06 mmol/L) cholesterol (data referred as mean difference of the differences in each study between cocoa group and control).

Hartley *et al.*[46] carried out an analysis of RCTs lasting al least 3 months which investigated the effects of black or green tea or tea extracts involving healthy adults or those at high risk of CVD. The global analysis of the consumption of black tea (1 g extract/day, 1,29 g black tea polyphenols/day; three serving of black tea (200 mL/serving) and 318 mg black tea catechins/day) was found to produce statistically significant reductions in LDL -C (mean difference -0.43 mmol/L, 95% CI -0.56 to -0.31). Green tea (58.91 mg catechin in green tea, 500 mg green tea polyphenols/day, 375 mg green tea extract/day, 200 mg theanine and 400 mg decaffeinated catechin green tea extract/day) was also found

to produce statistically significant reductions in total cholesterol (mean difference MD -0.62 mmol/L, 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 were analyzed together they showed favorable effects on LDL-C (MD - 0.48 mmol/L, 95% CI -0.61 to -0.35).

The meta-analysis of Desch *et al.*[47] and Hooper *et al.*[45] confirmed the blood pressure-lowering capacity of flavanol-rich cocoa products. Desch *et al.*[47] analyzed 297 participants including six cross-over and four parallel-group designs. Although the studies displayed a diverse spectrum of treatment regimens (duration from 2 to 8 weeks and intake (from 6.8 mg/d- 902 mg/d flavanol), results revealed that the mean blood pressure reduction was -4.5mmHg (95% CI -5.9 to -3.2; I2=89%) for systolic BP and -2.5mmHg (95% CI -3.9 to -1.2; I2=90%) for diastolic BP. Hooper *et al.*[45] reviewed the effects of chocolate, cocoa, and flavan-3-ols including 42 acute or short-term chronic (≤18 wk) RCTs that comprised 1297 participants. They observed reductions in diastolic BP (-1.60 mm Hg; 95% CI: -2.77, -0.43 mm Hg) and mean arterial pressure (-1.64 mm Hg; 95% CI: -3.27, -0.01 mm Hg). Although some studied did not identify dose-dependent effects of ECG, subgrouping by ECG dose suggested greater effects for systolic and diastolic BP at doses >50 mg/d. In the above reported meta-analyses by Hibi *et al.*[38] a significant decrease of systolic BP compared to baseline was observed only in the high catechin group (-1.1mmHg, 95%CI: -2.1 to -0.1), ($P < 0.01$). The 11 RCTs analysed by Hartley *et al.*[46] evidenced that black tea consumption significantly reduced systolic BP (MD -1.85 mmHg,95%CI -3.21 to -0.48), and green tea consumption significantly decreased both systolic and diastolic BP (MD -3.18 mmHg, 95% CI -5.25 to -1.11 and MD -3.42, 95% CI -4.54 to -2.30, respectively).

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

Analysis in endothelial cells show the enhancement of nitric oxide production by EGCG[48, 50]. Apart from the metabolic regulation, recent studies are focusing on assessing the epigenetic modulation of candidate genes of MetS by flavan 3-ols[48].

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

Beta-glucan

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

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

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

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

called for well controlled intervention studies[57]. A meta-analysis of randomised controlled trials showed that oat BG at doses higher than 3 g/day reduced LDL-C and total cholesterol significantly compared to control, with little or no effect on HDL-C and TG irrespective of dose or study duration[58]. The authors specified that the effectiveness of oat BG is linked to its high molecular weight and associated physicochemical properties, however called for more dose response and longer studies to evaluate impacts of chronic consumption of oat BG in healthy and MetS populations.

Ibrugger *et al.*[59] compared the effects of BG from oats and barley and showed that neither affected blood lipids significantly compared to the control. However, the consumption of 3.3 g/day oat BG led to the largest observed decrease in total cholesterol and LDL-C, as well as significantly reducing TG. The authors identified a lack of systematic studies, with great differences amongst studies in terms of study foods, dose and study duration. Few of the intervention studies investigated the dose-effect relationship between oat BG and blood cholesterol. Biorklund *et al.*[60] reported that consuming a drink containing 5g/day oat BG resulted in a 6.7% decrease in LDL-C, while consumption of the drink containing 10g/day oat BG reduced LDL-C by only 3.7%, compared to control drink. Kerckhoffs *et al.*[61] highlighted that processing of oats could have an adverse effect on the cholesterol lowering effect. Charlton *et al.*[62] showed that 1.5 g/day provided as cereal flakes was just as effective as 3 g/day provided as porridge in lowering blood cholesterol. Wolever *et al.*[63] showed the importance of molecular weight for the effectiveness of oat BG towards cholesterol markers[63]. The impact of processing on oat BG properties has been recently reviewed by Grundy *et al.*[64]. In healthy people, BG consumption does not appear to affect lipid homeostasis[65].

Epidemiological studies have supported the association between whole grain intake and improved metabolic risk factors for type 2 diabetes and metabolic syndrome[66, 67]. The fasting glucose concentrations decreased across increasing quartile categories of whole-grain intake. However, few clinical trials have focused on the impact of the consumption of BG on glucose metabolism. Many studies investigating BG and lipid homeostasis have also investigated impacts on glucose homeostasis. The EFSA panel supported a claim that consuming 4g of BG from oats or barley for each 30g of available carbohydrate decreased post-prandial glycaemic response without disproportionally increasing insulin response. The effect was observed when BG was incorporated into carbohydrate-rich food (e.g. bread or pasta) and when combined into a meal[55]. Consuming at least 4g BG per meal, from either oats or barley, and where the BG is soluble and has a MW >250 000 g/mol is sufficient to significantly reduce post-prandial area under curve (AUC) by 27±3 mmol·min/l for meals with ~30–80g of available carbohydrates[68]. He *et al.*[69] carried out a meta-analysis of controlled intervention trials, and showed that consumption of either wholegrain oats or BG extracted from oats was associated with strong significant reducing effects on fasting

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

Few clinical trials have specifically studied the effects of the consumption of BG on blood pressure. The results of the different studies show discrepancies. Past results obtained with healthy volunteers generally did not demonstrate an effect of the consumption of fibres on blood pressure compared to low-fibre grain supplementation[71]. However, a recent meta-analysis concluded that systolic and 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 to 2.7 mmHg) respectively by diets rich in BG, for a median difference in BG of 4 g in healthy volunteers[72]. The consumption of BG should thus help to manage BP of non-healthy people, especially people at risk of MetS. In 2006, Behall *et al.*[73] demonstrated the effects of consuming controlled portions of whole-grain rice and barley BG on BP in 25 overweight/obese mildly hypercholesterolemic women. Both wholegrain rice and barley BG interventions led to significant decreases in diastolic BP and the mean arterial pressure, especially in post-menopausal women. In a randomized cross-over design, the consumption of a diet enriched in legumes and barley by overweight women for 4 weeks induced a significantly reduction (−3 %, P< 0·05) of the diastolic BP but no effect was observed on systolic BP compared to the equivalent diet without legumes and barley[74]. A similar observation was made in healthy and obese men and women consuming multifunctional diets that included BG amongst other health enhancing constituents[53]. However, it is difficult to dissociate the effect of BG from other constituents in the diet.

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

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

n-3 long chain polyunsaturated fatty acids

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Foods with a combination of high-oleic acid canola oil-DHA (HOCO-DHA) and barley BG have been used in the CONFIDENCE trial, a randomized, single-blind crossover trial with four treatment phases of 28 days each[95]. The possible synergism between DHA and other bioactives was also in the focus of the EU project PATHWAY-27 (Pivotal assessment of the effects of bioactives on health and wellbeing. From human genoma to food industry)[96] that investigated the role and mechanisms of action of DHA, oat BG, and AC, alone and in combination, in the counteraction of MetS considering them not as stand-alone molecules but as ingredients of food. In PATHWAY-27, three monocentric, parallel-arm, randomized, double blind pilot trials and a multicentre, randomized, placebo-controlled, parallel-arm dietary intervention study were performed on subjects at risk of MetS. At present, neither CONFIDENCE nor PATHWAY-27 results are available in the literature to report on the outcomes of potential combined effects in interventions involving DHA, BG (and AC).

Based on available results, the increased intake of n-3 LCPUFA seem to have an effect on BW and BMI only if it is associated to modification of the whole diet so we can argue that it is not simply due to LCPUFA themselves. The effectiveness of n-3 LCPUFA on other parameters has been evidenced in trials using both supplements and enriched food with differences related to the daily dose, the duration of the intervention, and the EPA:DHA ratio.

Conclusion

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

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

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

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

PATHWAY-27 consortium published scientific guidelines to guide the scientific community to design trials for bioactive effectiveness[97].

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Conflict of interest

The Authors declare no conflict of interest.

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