Serial Review

Health Beneficial Effects of Food Factors Can Be Applicable to Humans?

Guest Editor: Kazuki Kanazawa

Flavan 3-ols improve metabolic syndrome risk factors: evidence and mechanisms

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Flavan 3-ols, a type of polyphenolic substance, are distributed in a number of plant foods. Of these foods, chocolate is very rich in flavan 3-ols as flavan 3-ol monomers, (+)-catechin and (–)-epicatechin, and the oligomers as procyanidins. There is evidence that cacao products containing flavan 3-ols have the potential to contribute to the risk reduction of cardiometabolic disorders according to recent epidemiological or intervention studies. This review focuses on recent advances in research on the ability of flavan 3-ols to reduce the risk of cardiovascular disease as a result of improving metabolic syndrome risk factors and these mechanisms.

***Key Words*: flavan 3-ols, chocolate, cardiovascular diseases, metabolic syndrome, risk factors**

Flavan 3-ols, a type of polyphenolic substance, are distributed in a number of plant foods and supplements such as cacao beans, red wine, beer, berries, apples, black soy bean and French maritime pine bark. Of these foods, chocolate is the most abundant flavan 3-ols containing food. As shown in Fig. 1, these include the flavan 3-ol monomers, (+)-catechin and (−)-epicatechin, and the oligomers, B-type flavan 3-ols, such as procyanidin B2 (dimer), procyanidin C1 (trimer), and cinnamtannin A2 (tetramer) that are linked by C4–C8 bonds.[1–3] It has been reported that chocolate contains oligomers ranging from dimers to decamer flavan 3-ols.[4] Recent studies have suggested that chocolate or flavan 3-ols have a positive influence on human health, due to antioxidant,[5,6] anti-inflammatory,[7,8] and anti-thrombotic effects.[9] There is also evidence that cacao products containing flavan 3-ols have the potential to contribute to the prevention of cardiometabolic disorders.[10] This review focuses on recent advances in research on the ability of flavan 3-ols to reduce the risk of cardiovascular disease as a result of improving metabolic syndrome risk factors.

Inverse Association of Chocolate Intake and the Risk of Developing Cardiovascular Diseases

The Kuna, an indigenous group who lives predominantly on small islands off the coast of Panama consume a large amount of natural cocoa drinks, and are nearly free of hypertension and cardiovascular disease. In contrast, Kuna who migrate to Panama urban sites lose this advantage, as they are no longer able to maintain their habit of drinking cocoa.[11] Recent epidemiological evidence suggests that ingestion of flavan 3-ols monomers reduces the risk of coronary heart disease.[12,13] These reports showed a strong inverse association between intake of (+)-catechin and (−)-epicatechin as flavan 3-ols monomers and death from coronary heart disease. Epidemiological evidence also suggests that ingestion of chocolate reduces the risk of cardiovascular diseases such as stroke and cardiometabolic diseases.[14,15] Buitrago-Lopez *et al.*[14] using six cohort studies and one cross-sectional study showed that the highest level of chocolate consumption was associated with a 37% reduction in cardiovascular disease (relative risk 0.63, 95% CI 0.44–0.90) and a 29% reduction in stroke compared with the lowest levels (Fig. 2). Larsson *et al.*[15] also reported a meta-analysis of 5 studies that showed the multivariable relative risk of stroke was 0.83 (95% CI 0.70–0.99) for the highest quartile of chocolate consumption (median 62.9 g/week) compared with the lowest quartile (median 0 g/week). Based on observational evidence, these results suggested that the level of chocolate consumption was associated with a substantial reduction in the risk of cardiovascular disorders.

Association of Ingestion of Chocolate and Metabolic Syndrome Risk Factors

Numerous randomized, controlled trials (RCT) have investigated the effects of chocolate or cocoa products, especially dark chocolate, on metabolic syndrome risk factors such as hypertension,[16,17] vascular endothelial dysfunction,[18,19] dyslipidemia,[20,21] and glucose intolerance.[22,23] As shown in Table 1, seven meta-analyses of chocolate intervention trials[24–30] have been reported recently. Hooper *et al.*[30] analyzed the data of 1297 subjects in 42 acute or short-term chronic RCTs and showed that insulin resistance (HOMA-IR: −0.67; 95% CI: −0.98, −0.36) was improved by consumption of chocolate or cocoa due to significant reductions in serum insulin. They also reported that flow-mediated dilatation (FMD) improved after chronic (1.34%; 95% CI: 1.00%, 1.68%) and acute (3.19%; 95% CI: 2.04%, 4.33%) chocolate ingestion. Reductions in diastolic blood pressure (BP; −1.60 mmHg; 95% CI: −2.77, −0.43 mmHg) and mean arterial pressure (−1.64 mmHg; 95% CI: −3.27, −0.01 mmHg), and marginally significant improvements in LDL (−0.07 mmol/l; 95% CI: −0.13, 0.00 mmol/l) and HDL cholesterol levels (0.03 mmol/L; 95% CI: 0.00, 0.06 mmol/l) were also observed in the study. These data are consistent with the beneficial effects of cocoa products on metabolic syndrome risk factors shown in

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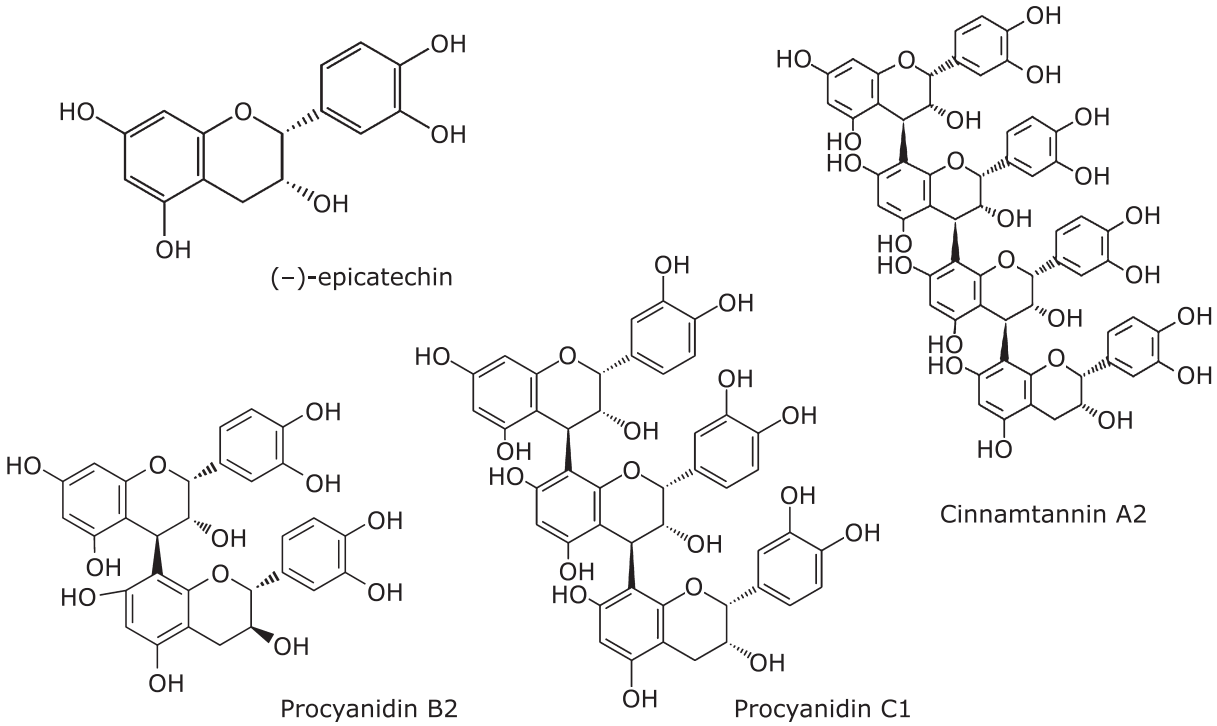

Fig. 1. Chemical structures of flavan 3-ols in chocolate.[1–3]

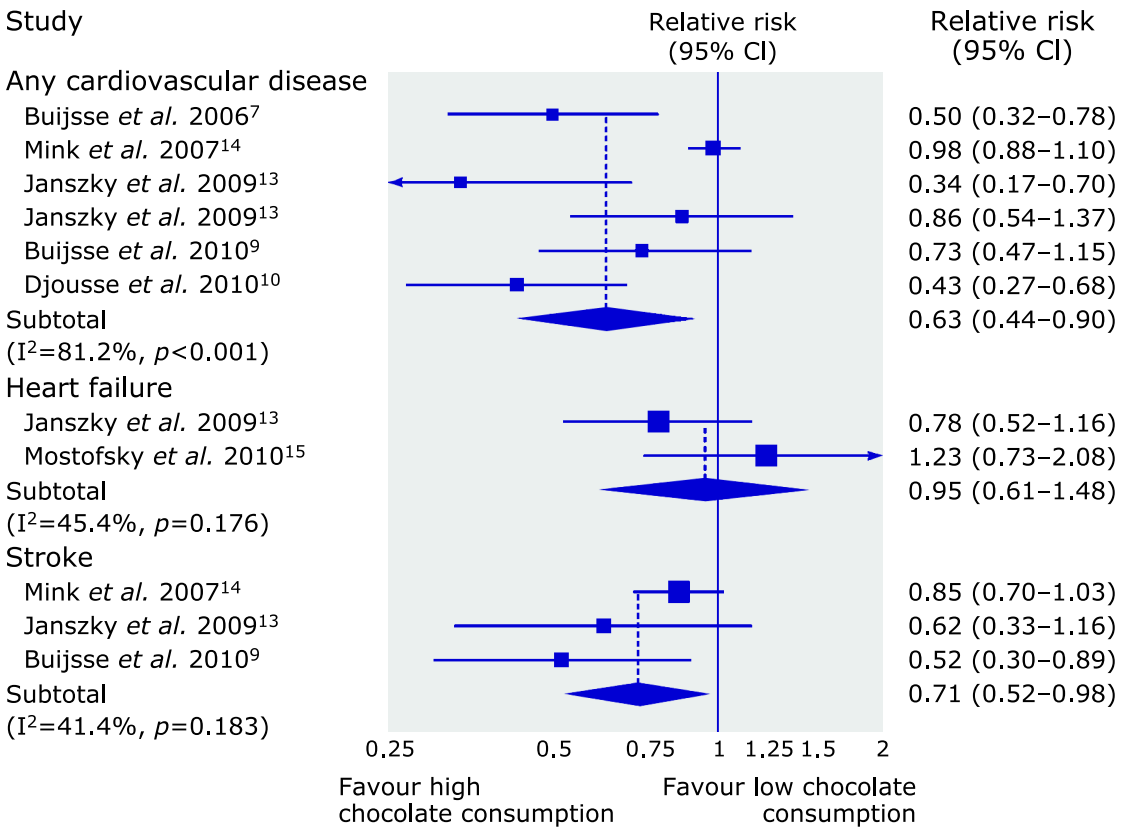

Fig. 2. Relative risks for cardiovascular disease, heart failure, and stroke in adults with higher levels of chocolate consumption compared with lower levels. Reproduced from (14) with permission.

Table 1. Effect of chocolate on cardiovascular health: systematic reviews and meta analyses

			n (study)	*n* (subjects)	
Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, Cassidy A.[30]	*Am J Clin Nutr* 2012; **95**: 740–751.	Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials	42	1297	HOMA-IR: –0.67; 95% CI: –0.98, –0.36 chronic FMD: 1.34%; 95% CI: 1.00%, 1.68% acuteFMD: 3.19%; 95% CI: 2.04%, 4.33% MBP: –1.64 mmHg; 95% CI: –3.27, –0.01 mmHg LDL: –0.07 mmol/l; 95% CI: –0.13, 0.00 mmol/l HDL: 0.03 mmol/l; 95% CI: 0.00, 0.06 mmol/l
Shrime MG, Bauer SR, McDonald AC, Chowdhury NH, Coltart CE, Ding EL.[29]	*J Nutr* 2011; **141**: 1982–1988.	Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies	24	1106	SBP: –1.63 mmHg ($p = 0.033$) LDL: –0.077 mmol/l ($p = 0.038$) HDL: 0.046 mmol/l ($p = 0.037$) HOMA-IR: –0.94 points ($p<0.001$) FMD: 1.53% ($p<0.001$)
Tokede OA, Gaziano JM, Djoussé L.[28]	*Eur J Clin Nutr* 2011; **65**: 879–886.	Effects of cocoa products/dark chocolate on serum lipids: a meta-analysis	10	320	TC: –6.23 mg/dl (–11.60, –0.85 mg/dl) LDL: –5.90 mg/dl (–10.47, –1.32 mg/dl) HDL: –0.76 mg/dl (–3.02–1.51 mg/dl) TG: –5.06 mg/dl (–13.45–3.32 mg/dl)
Ried K, Sullivan T, Fakler P, Frank OR, Stocks NP.[27]	*BMC Med* 2010 Jun 28; **8**: 39.	Does chocolate reduce blood pressure? A meta-analysis	13	288	SBP: –3.2 +/– 1.9 mmHg ($p = 0.001$) DBP: –2.0 +/– 1.3 mmHg ($p = 0.003$)
Jia L, Liu X, Bai YY, Li SH, Sun K, He C, Hui R.[13]	*Am J Clin Nutr* 2010; **92**: 218–225.	Short-term effect of cocoa product consumption on lipid profile: a meta-analysis of randomized controlled trials	8	215	TC: 5.82 mg/dl (95% CI: –12.39, 0.76; $p = 0.08$) LDL: –5.87 mg/dl (95% CI: –11.13, –0.61; $p<0.05$)
Desch S, Schmidt J, Kobler D, Sonnabend M, Eitel I, Sareban M, Rahimi K, Schuler G, Thiele H.[26]	*Am J Hypertens* 2010; **23**: 97–103.	Effect of cocoa products on blood pressure: systematic review and meta-analysis	10	297	MBP: –4.5 mmHg (–5.9–-3.2, $p<0.001$) SBP: –2.5 mmHg (–3.9–-1.2, $p<0.001$)
Taubert D, Roesen R, Schömig E[25]	*Arch Intern Med* 2007; **167**: 626–634.	Effect of cocoa and tea intake on blood pressure: a meta-analysis	5	173	MBP: –4.7 mmHg (–7.6–-1.8 mmHg; $p = 0.002$) SBP: –2.8 mmHg (–4.8–-0.8 mmHg; $p = 0.006$)

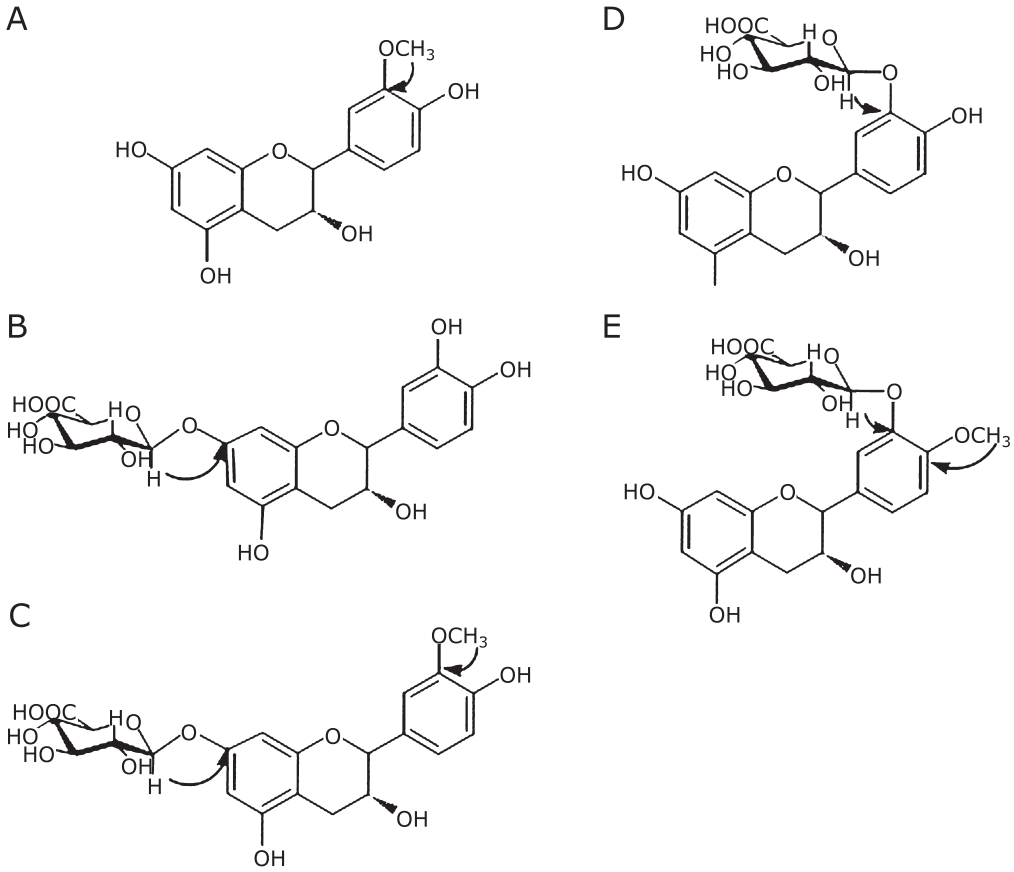

Fig. 3. Structure of (–)-epicatechin metabolites. (A) 3'-*O*-Methyl-(–)-epicatechin, (B) (–)-epicatechin-7-*O*-glucuronide, (C) 3'-*O*-Methyl-(–)-epicatechin-7-*O*-glucuronide, (D) (–)-epicatechin-3'-*O*-glucuronide, (E) 4'-*O*-Methyl-(–)-epicatechin-3'-*O*-glucuronide. Chemicals A, B and C were obtained from rat urine, D and E were obtained from human.

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Table 2. Chololate consumption frequency predicts lower BMI: regression results[45]

Adjustment model	Chocolate consumption frequency, association with BMI	
	δ (SE)	*p* value
Unadjusted	–0.142 (0.053)	0.08
Age and sex adjusted	–0.126 (0.053)	0.02
Age, sex and activity adjusted	–0.130 (0.052)	0.01
Age, sex and calorie adjusted	–0.146 (0.059)	0.01
Age, sex and satfat adjusted	–0.190 (0.059)	0.001
Age, sex, satfat and CES-D adjusted	–0.191 (0.059)	0.001
Age, sex, satfat, fruite and vegetable, and CES-D adjusted	–0.201 (0.060)	0.001
Age, sex, satfat, fruite and vegetable, and CES-D and calories adjusted	–0.208 (0.060)	0.001

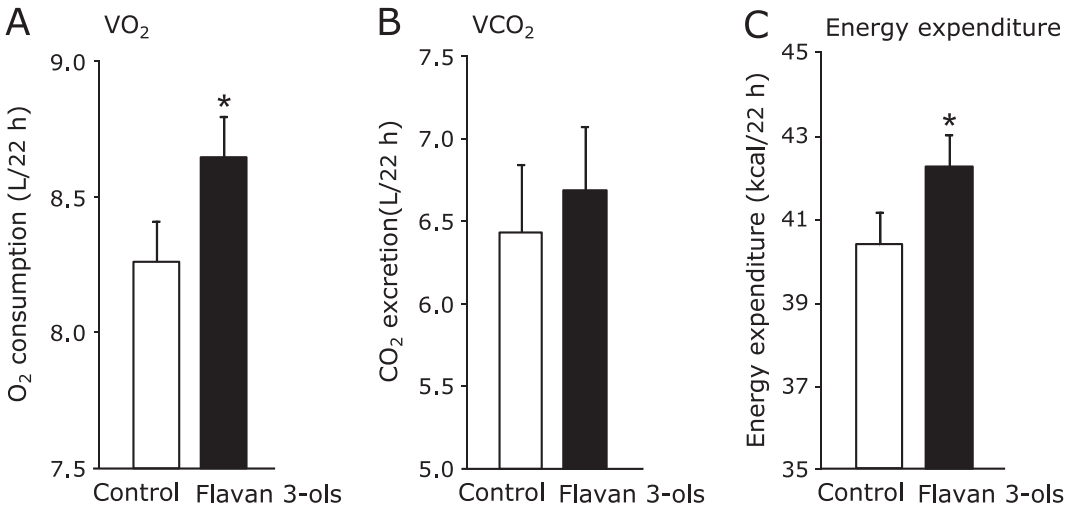

Fig. 4. VO_2 (A), VCO_2 (B) and energy expenditure (C) in rats fed control or 0.2% flavan 3-ols containing diet. Values are mean and SD. Significantly different from control, *p<0.05.

short-term intervention trials. However, further larger and longer-duration trials are required to confirm the potential cardiovascular benefits of cocoa flavan-3-ols.

Bioavailability of Flavan 3-ol

Numerous reports have investigated the bioavailability of flavan 3-ols. Flavan 3-ols monomers, such as (−)-epicatechin and (+)-catechin are well absorbed, and are metabolized mainly in the small intestine or liver, forming sulfate, glucuronide or methylated metabolites through the action of sulfotransferases (SULT), uridine-5'-diphosphate glucuronosyltransferases (UGTs) and catechol-*O*-methyltransferases (COMT),[31] respectively. Non-metabolized flavan 3-ol monomers are therefore rarely detected in the blood. We provided evidence that the chemical structure of (−)-epicatechin glucuronide, a major metabolite of (−)-epicatechin, was different between human and rats(Fig. 3).[32] The antioxidative activities of those metabolites was also shown to be reduced in metabolites derived from human biomaterials.[33]

In contrast, there are numerous feeding studies on animals and humans that demonstrate polymeric epicatechin as procyanidins are not absorbed.[34] For example, we showed that only about 0.5% of the epicatechin dimer, procyanidin B2, is absorbed,[35] with the majority passing unaltered into the large intestine where it is catabolized by colonic microflora to a diverse range of phenolic acids[36,37] including 3-(3-hydroxyphenyl)propionic acid and 4-*O*-methylgallic acid.[38] These acids are then absorbed into the circulatory system and excreted in the urine. It is possible the biological effects of procyanidins described above are attributable to these phenolic acids, although there is a lack of detailed information in this area.

Flavan 3-ols Bioactivity: *In Vitro* Studies

As described above, despite the bioavailability of flavan 3-ols being very low, there has been a large number of *in vitro* studies to examine improvements in metabolic syndrome risk factors following the ingestion of these compounds. Studies using cell culture or isolated organs showed that the nitric oxide (NO) radical, a potent endothelium dilatation factor, and endothelial nitric oxide synthase were increased by the addition of flavan 3-ols.[39–41] However, almost all these investigations lacked physiological significance as the parent compounds rather than the metabolites were used at high levels than those achieved in blood following oral administration of flavan 3-ols. Several recent studies have investigated flavan 3-ols-conjugated metabolites in mammals and microbial degradation products, with one study showing that *O*-methylated epicatechin inhibited NADPH oxidase in the endothelium.[42] Phenolic acids, which are metabolites of colonic fermentation, have also been reported to possess certain bioactivities.[43,44] Unfortunately, biological significance was also not achieved in these studies due to the high dose of metabolites used in the experiments. Taken together, these studies suggest that absorbed procyanidins, catechins or phenolic acids contributed only a portion of the improvement in metabolic syndrome risk factors.

Table 3. Influence of single oral administration of cocoa or flavan 3-ols on microcirculation in rat cremaster muscle

	n		ΔRBC velocity (μm/s)	Δnewly recruited capillary (number)	Δheart Rate (beats/min)	Δmean blood pressure (mmHg)
Vehicle	8	5 min	1.2 ± 2.6	4.3 ± 0.8	–2.1 ± 1.8	–4.6 ± 4.3
		20 min	–0.6 ± 3.1	8.6 ± 3.1	3.2 ± 8.2	2.1 ± 3.6
Cocoa	8	5 min	61.2 ± 23.3**	8.2 ± 1.3	7.3 ± 3.6	14.2 ± 9.6
		20 min	116 ± 26.2**	19.6 ± 5.6**	12.2 ± 4.2*	30.2 ± 7.8*
Flavan 3-ols	8	5 min	58.4 ± 29.7**	7.6 ± 1.4	6.3 ± 2.8	13.8 ± 8.3
	8	20 min	98.6 ± 35.6**	19.1 ± 4.2**	14.6 ± 3.1*	28.9 ± 8.8*

Each value represnts the mean ± SD. Significantly difference from vehicle; *p<0.05, **p<0.01.

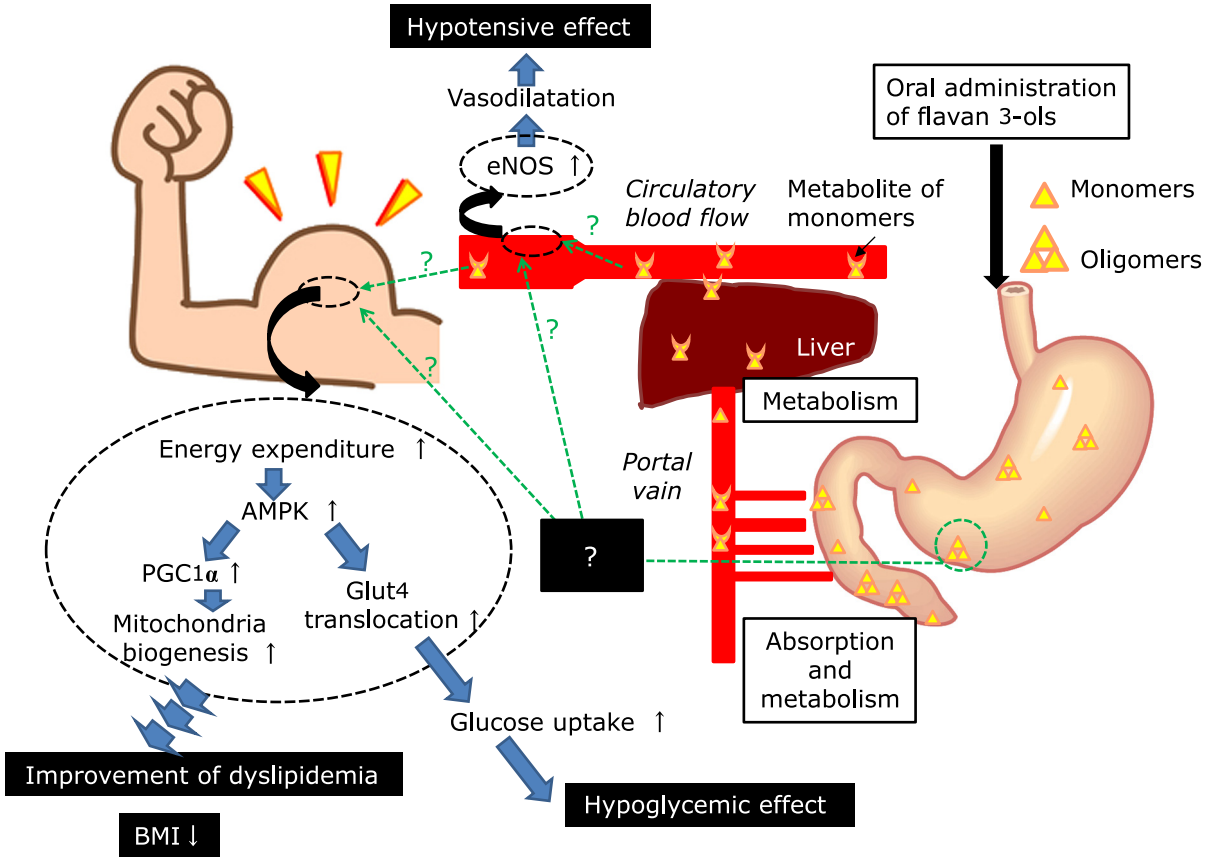

Fig. 5. The results indicated these recent reports were summarized as Table 5. Hypotensive effect was shown by oral administrated flavan 3-ols through induced endothelial nitrogen oxide synthase (eNOS) expression. In skeletal muscle, enhancement of energy expenditure was induced by oral administration of flavan 3-ols, it resulted activation of AMPK. AMPK activation enhanced both transcription and translocation of glucose transporter type 4 (GLUT4), resulting acceleration of glucose uptake. AMPK might be activated peroxisome-proliferator- activated receptor coactivator 1 (PGC1α) which was the key factor of mitochondrial biogenesis. Improvement of dyslipidemia or BMI lowering activity seen in RCT or epidemiological studies also might be induced by such mitochondria biogenesis promoting effect.

Flavan 3-ols Bioactivities—a New Angle of Observation

A study in over 1000 American men and women showed a negative correlation between the frequency of chocolate consumption and body mass index (BMI) (Table 2).[45] Taub *et al.*[46] also reported that ingestion of chocolate stimulated mitochondrial biogenesis of skeletal muscle in patients with type 2 diabetes or heart failure. We showed recently that repeated ingestion of the flavan 3-ols fraction influenced energy expenditure in rats.[47] In that study, the animals were fed for 2 weeks with either a normal diet or one containing 0.2% flavan 3-ols derived from cacao. At the end of the experimental period, energy expenditure was estimated by an indirect calorimetric method that measured oxygen consumption (VO_2) and carbon dioxide excretion (VCO_2) for 22 h. As shown in Fig. 4, total O_2 consumption was increased significantly in the flavan 3-ols group compared with controls. As a consequence, total energy expenditure also increased significantly in the flavan 3-ols group. We observed that repeated ingestion of flavan 3-ols reduced mean blood pressure to the same degree as that reported in published meta-analyses. In contrast, a single administration of flavan 3-ols in rats was shown to cause an immediate elevation in blood pressure and heart rate leading to increased blood flow and recruitment of capillaries in skeletal muscle (Table 3)[48]. In addition, studies by Yamashita *et al.*[49] demonstrated that flavan 3-ols prevented glucose intolerance and obesity by promoting translocation of glucose transporter

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4 and phosphorylation of AMP-activated protein kinase (AMPK) in the plasma membrane of skeletal muscle and brown adipose tissues.

These results in recent reports are summarized in Fig. 5. A hypotensive effect is produced by the oral administration of flavan 3-ols that induced expression of endothelial nitrogen oxide synthase (eNOS), while this effect is unclear in a point whether this effect was produced by metabolites of monomers in circulating blood or oligomers that remained in the gastrointestinal tract. In skeletal muscle, enhancement of energy expenditure is induced by oral administration of flavan 3-ols following with AMPK activation. AMPK activation enhanced both transcription and translocation of glucose transporter type 4 (GLUT4), resulting in acceleration of glucose uptake. It has been shown that AMPK also activates peroxisome-proliferator-activated receptor coactivator 1(PGC1α) which is a key factor in mitochondrial biogenesis. Improvement of dyslipidemia or lowering of BMI in RCT or epidemiological studies may also be induced by this mitochondrial biogenesis promoting effect. On the basis of these results, recent studies have attempted to define the mechanism responsible for the beneficial effects of flavan 3-ols from a new perspective.

Conclusion

In conclusion, flavan 3-ols may improve hypertension, dyslipidemia, insulin resistance, and obesity induced by inappropriate daily habits. However, further studies are required to elucidate the mechanisms responsible for the risk reduction of cardiovascular diseases caused by flavan 3-ols.

Conflict of Interest

No potential conflicts of interest were disclosed.

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