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Special Article

Dietary modulation of large extracellular vesicles: the good and the bad for human health

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Abstract

Extracellular vesicles encompassing nanovesicles derived from the endosome system and generated by plasmatic membrane shedding, become of increasing interest in view of their ability to sustain a cell-to-cell communication and with the possibility to be used as surrogate biomarkers of healthy and unhealthy trajectories. Over the last decades, nutritional strategies have been developed to preserve health and their impact on circulating EVs is arousing a growing interest. Data available from published studies are now sufficient for a first integration in order to better understand the role of EVs in the relation between diet and health. Thus, this review focuses on human intervention studies investigating the impact of diet or its components on circulating EVs. Due to analytical bias, only large EVs have been assessed so far. The analysis highlights that poor-quality diets with elevated fat and sugar content increase levels of circulating large EVs that can be partly counteracted by healthy food or some food micronutrients and bioactive compounds. However, knowledge on the content and the biological functions of these diet-induced EVs are still missing. It is of crucial importance to address these aspects in new research in order to state on EVs as mediators of the effects of diet on health.

Keywords

Extracellular vesicles, microparticles, exosomes, diet, dietary constituents, human intervention studies, risk factor, nutritional biomarkers

Introduction

Dietary pattern is an important driver of non-communicable diseases (NCDs) such as obesity, diabetes and metabolic syndrome leading to cardiovascular diseases (CVD). In western societies, individuals are routinely exposed to postprandial lipaemia, hypertriglyceridaemia and hyperglycaemia that have been shown to be risk factors for CVD^{1, 2} including coronary artery diseases (CAD)^{3, 4}, and that contribute to the development of atherosclerosis^{5, 6}. These metabolic changes induce particularly the activation of circulating immune cells and endothelial cells, one feature of which is the increased release

of circulating extracellular vesicles (EVs)^{7, 8}. EVs, including small EVs (previously called exosomes ; S-EVs) and large EVs (known as microparticles; L-EVs), are shed membrane particles of less than 1µm in diameter that emerged as candidate meaningful biomarker for the diagnosis and the prognosis of cardiometabolic disorders⁹⁻¹⁴. S-EVs are vesicles of endo-lysosomal origin that are released as a consequence of the fusion of the multivesicular endosome (namely MVB) with the plasma membrane, whereas L-EVs are generated by budding and fission of the plasma membrane⁸. Both EV subtypes convey proteins, nucleic acids and lipids with the possibility to be used as markers and conferring to EVs the ability to sustain a cell-to-cell communication. As an example, circulating endothelial L-EVs and their increased level in plasma assessed at fasting state are both emerging biomarkers and active effectors of endothelial dysfunction in CVD¹⁵⁻¹⁷. Similarly, Crewe et al.¹⁸ demonstrated that S-EVs are produced locally by endothelial cells in the adipose tissue in fasting mice, but not after feeding. In addition to demonstrate that EVs are regulated by fasting/refeeding, the authors suggest that EVs participate the tissue response to metabolic changes in the systemic nutrient state.

Since the 2000s, the relationship between diet and circulating EVs has been the subject of numerous studies. Dietary interventions in animal models have demonstrated that chronic high-energy diet (e.g. high fat or high carbohydrate diet) results in an increased level of circulating S-¹⁹ and L-EVs²⁰⁻²². Inversely, plant-based diets are highly protective for health²³. This is partly attributed to EV-associated plant bioactive compounds, especially polyphenols with a body of evidence in preventing cardiometabolic diseases especially through the reduction of vascular dysfunctions associated with ageing and unbalanced nutrition²⁴⁻²⁶. In addition to improve endothelial function, diet supplemented with polyphenols from red wine has been shown to lower the level of endothelial EVs circulating at fasting state in a rodent model of hypertension²⁷.

Interestingly, food such as fruits and vegetables and cow milk-based products^{28, 29} are dietary sources of exogenous EVs (mainly S-EVs) namely EV-like nanoparticles. As other EVs, they convey proteins, lipids and nucleic acids in a phospholipid bilayer, but they can also encompass nutrients and/or plant bioactive compounds such as polyphenols^{30, 31}. These S-EVs have been demonstrated to be

absorbed, detected in host tissues few hours after their intake. The biological effects of EV-like nanoparticles on the physiology of the consumer is a matter of concern.

The close relationship between diet and health, and the growing development of EV-based biomarkers in medicine make necessary to consider the modulation of EVs by the diet, both at fasting and postprandial states in humans. The present review focuses on human interventions investigating the impact of a single meal and of a chronic intake on circulating EVs. It also introduces how dietary compounds act to trigger physiological pathways controlling the biogenesis and/or the release of endogenous EVs. Finally, this review provides reports on the biological functions of diet-associated EVs and raises the need for further investigations in this domain, especially in order to provide an accurate phenotyping of diet-induced EVs.

1. Why human nutritional intervention studies focused on L-EVs?

Since the 2000s, 29 interventional studies have investigated the impact of diet and their constituents on circulating EVs in Humans. In these nutritional intervention studies, EVs were analysed on platelet-poor or platelet-free plasma (PPP or PFP) obtained by centrifugation of blood samples up to 17,000 *g*. This centrifugation pellets platelets but not the L-EVs and S-EVs that need longer centrifugation at 20,000 *g* or 100,000 *g* to be pelleted respectively³². In these PPP/PFP, EVs were detected by flow cytometry based on different combinations of constitutive and/or inducible surface markers (Table 1) after immunostaining. This strategy of analysis limits the detection and the quantification to specific EV subtypes. First, the conventional flow cytometers used in these studies (LSRII, FACS Aria, FACScan, Canto, FC500, FACSCalibur) are not sensitive enough to detect scattered light or emitted fluorescence from particles smaller than 300 nm in diameter, and therefore do not allow the direct detection of S-EVs^{33, 34}. In addition, plasma lipids (e.g. chylomicrons) due to their size may interfere with the detection of EVs by flow cytometry³⁵. To avoid this limit, a fluorescent staining mainly based on the detection of phosphatidylserine specifically expressed at the surface of L-EVs was used. Therefore, this explains why these nutritional intervention studies only report on the effect on L-EVs.

Above methodological aspects, the interest in L-EVs may be explained by a greater susceptibility of L-EVs to changes in response to a stimulus than S-EVs^{36, 37}. Moreover, the content in proteins of L-EVs was reported as closer to those of the donor cell than the proteome of S-EVs^{36, 38}, thus strengthening the interest for the L-EVs in human intervention studies. However, it is worth noting that the secretion, the content and the bioactivity of S-EVs have been reported in *in vitro* studies as modulated by nutritional factors such as hyperglycaemia³⁹⁻⁴¹ or polyphenol supplementations⁴²⁻⁴⁴.

Among the subpopulations of EVs present in plasma, endothelial L-EVs whose proportion is estimated to less than 5% are the most investigated ahead of platelet- and erythrocyte-derived L-EVs which account for 70%-95% and 8-16% of the total plasma L-EVs, respectively⁴⁵⁻⁴⁹. This large interest in endothelial L-EVs can be explained by the widely documented contribution of endothelial dysfunction in the development and the complications of cardiometabolic diseases⁵⁰. Whatever the subtype of EVs investigated, human intervention studies were controlled by ethical considerations that limit the number and volume of blood sampling and therefore that constrain studies to few timepoints after a nutritional intervention. By consequence, knowledge on the pharmacokinetic of EVs during the postprandial period is still incomplete. Cross-over and/or a placebo control designs have been used to ascertain the impact of dietary intakes on circulating L-EVs. They allowed to demonstrate first the variation of the circulating L-EVs during the postprandial period induced by an acute diet intake (Table 2) and secondarily the effect of chronic intakes on the basal circulating L-EVs at fasting (Table 3).

Cell source of large EVs (namely microparticles)	Cluster of differentiation staining	Protein Symbol	Full name
Endothelial MPs	CD31+ CD41- CD42a- CD42b- CD45- CD62b- CD62e+ CD105+ CD106 CD144+ CD146+	PECAM1 ITGA2B GP9 Gplb PTPRC SELP SELE ENG VCAM1 CDH5 MCAM	platelet and endothelial cell adhesion molecule 1 integrin subunit alpha 2b glycoprotein IX platelet glycoprotein Ib platelet protein tyrosine phosphatase receptor type C selectin P selectin E endoglin vascular cell adhesion molecule 1 cadherin 5 melanoma cell adhesion molecule
Platelet MPs	CD41+ CD42b+ CD61+ CD62b+ Fibrogen+ TF(CD142)+	ITGA2B Gplb ITGB3 SELP FGB F3	integrin subunit alpha 2b glycoprotein Ib platelet integrin subunit beta 3 selectin P fibrinogen beta chain coagulation factor III, tissue facto
Leukocyte MPs	CD4+ CD8 CD11a+ CD11b+ CD41- CD45+	CD4 CD8A ITGAL ITGAM ITGA2B PTPRC	CD4 molecule CD8a molecule integrin subunit alpha L integrin subunit alpha M integrin subunit alpha 2b protein tyrosine phosphatase receptor type C

Table 1: Cell surface markers used for the identification of EV subpopulations by flow cytometry.

114 **Table 2: Clinical trials investigating the impact of acute dietary intakes on EVs during the postprandial period.**

Publication	Study design	Country	Participants	Intervention	Investigated EVs	Main results
Bulut et al. J Cardiovasc Pharmacol 2013 51	Randomized Cross-over	EU (Germany)	Healthy men (n= 10) Age= 44.2 ± 9.6 BMI= 24.4 ± 2.5	A single meal of French fries and hot pork sausage with sauce (1,200 kcal: 50 % from Fat; 30 % CHO; 20 % Proteins) ± 200 mL water or 200 mL coca cola or 100 mL red wine/10 0mL water, or 36 mL liquor (38 vol %)/164 mL water	AnnexinV ⁺ L-EVs: ▪ CD31 ⁺ /CD42b ⁻ endothelial L-EVs ▪ CD31 ⁺ /CD42b ⁺ platelet L-EVs From platelet-poor plasma By flow cytometry (FACScalibur, BD)	<ul style="list-style-type: none"> • ↑ postprandial (water; 1h: +15 %, 2 h: +30 %) AnnexinV⁺/CD31⁺/CD42b^{-/+} L-EVs • Red Wine consumption: ↓ meal- induced AnnexinV⁺/CD31⁺/CD42b⁻ /+ L-EVs compared to water (-83 %) • Coca: ↑ meal-induced AnnexinV⁺/CD31⁺/CD42b^{-/+} L-EVs compared to water (+109 %)
Eichner et al. Nutrients 2019 52		NA (US)	Obese prediabetic (PD, n= 17) and NGT (n= 8) volunteers Male:Female= 4:13 (PD), 1:7 (NGT) Age= 53.5 ± 4.1 (PD), 50.1 ± 5.1 (NGT) BMI= 32.1 ± 1.6 (PD), 33.2 ± 1.4 (NGT)	Oral Glucose Tolerance Test (a 75 g glucose intake that induced a 136 mg/dl glycaemia at peak, i.-e. 2 h)	Annexin V ⁺ EVs: ▪ CD31 ⁺ /CD41 ^{+/+} platelet EVs ▪ CD45 ⁺ /CD41 ⁻ , CD45 ⁺ leukocyte EVs ▪ CD105 ⁺ , CD31 ⁺ /CD41 ⁻ Endothelial EVs from Platelet-free plasma (17,000 g) by Flow cytometry (ImageStream MKII Amnis)	<ul style="list-style-type: none"> • ↓ total EVs • ↓ CD31⁺/CD41⁺ PEVs • ↓ CD31⁺ EVs • Ø CD45⁺ LEVs
Ferreira et al. Circulation 2004 53	Cross-over	NA (US)	Healthy volunteers (n= 18) Male:Female= 10:8 Age= 26 ± 3.8 BMI= 23 ± 2.3	I= High-fat meal (HF; 900 cal: 19 % from fat) C= Low-fat meal (LF; 900 cal: 0 % cal from fat)	<ul style="list-style-type: none"> ▪ CD31⁺/CD42b⁻ endothelial L-EVs ▪ CD31⁺/CD45⁺ leukocyte L-EVs From platelet-poor plasma (1,000 g) By flow cytometry (Coulter EPICS XL, Beckman)	<ul style="list-style-type: none"> • C= Ø effect of single Low-fat meal on postprandial E L-EV levels • I= ↑ postprandial (1 h: +39 %, 3 h: +70 %) CD31⁺/CD42b⁻ Endothelial L-EVs
Harrison et al. Eur J Appl Physiol 2009 54	Randomized Controlled	EU (Ireland)	Healthy active men (n= 8) Age= 26.9 ± 4.1 BMI= 26.0 ± 3.6	Oral fat tolerance test meal (1,450 kcal per 2 m ² body surface: 60 % from fat, 34 % from CHO) preceded by a rested time or a prolonged exercise (EXC)	CD31 ⁺ /CD42b ⁻ endothelial L-EVs from Platelet-poor plasma (1,000 g) by Flow cytometry (FACScalibur, BD)	<ul style="list-style-type: none"> • Test meal induces ↑ CD31⁺/CD42b⁻ endothelial L-EVs from 2h to 6h after intake (+160 %) • Ø of prior EXC on diet-induced EVs
Jenkins et al. J Physiol 2011 55	Cross-over	NA (US)	Healthy active men (n= 10) Age= 27 ± 0.9 BMI= 24.6 ± 0.7	A single high-fat meal portioned to 386 g/m ² body surface area (386 g= 1,362 kcal, 84 % from fat) ± prior endurance exercise	<ul style="list-style-type: none"> ▪ CD31⁺/CD42b⁻ endothelial L-EVs ▪ CD62E⁺ endothelial L-EVs From platelet-poor plasma (1,500 g) By flow cytometry (LSRII, BD)	<ul style="list-style-type: none"> • Ø effect of single high-fat meal on postprandial (4 h) endothelial L- EVs • Prior endurance exercise ↓ CD31⁺/CD42b⁻ and CD62E⁺ endothelial L-EVs (-50 %)

Publication	Study design	Country	Participants	Intervention	Investigated EVs	Main results
115 Table 2 (To be continued)						
Michelsen et al. Thrombosis Res 2009 56	Controlled	EU (Norway)	Carotid atherosclerosis patients (n= 40, CAP) and matched healthy volunteers (n= 20, H) Male:Female= 19:21 (CAP) 12:8(H) Age= 65.4-72.8 (CAP), 66.6-70.1(H) BMI= 26.4-28.8 (CAP), 24.8-28.4(H)	Standardized tolerance fat test consisting in sour cream porridge providing 70 % of calories from fat (66 % saturated, 32 % monosaturated, 2 % PUFAs)	CD41 ⁺ /CD61 ⁺ platelet L-EVs From platelet-free plasma (11,000 g) By flow cytometry (Canto, BD)	↑ postprandial (4 h) total CD41 ⁺ /CD61 ⁺ L-EVs (+49 %)
Phang et al. JNB 2012 57	Controlled Single blinded	South Pacific (Australia)	Healthy volunteers (n= 30) Male:Female= 15:15 Age= 43.7 ± 1.5 BMI= 26.1 ± 0.6	A single dose of sunola oil (C), 1 g EPA (EPA:DHA= 5:1) or 1 g of DHA (EPA:DHA= 1:5)	AnnexinV ⁺ /CD41a ⁺ platelet L-EVs From platelet-free plasma (3,000 g) by flow cytometry (Canto, BD)	<ul style="list-style-type: none"> • At baseline, Ø effect gender on the number of platelet L-EVs • 24h after a single dose of supplementation: Ø effect on AnnexinV⁺/CD41a⁺ MP levels • Only EPA supplementation ↓ thrombin generation mediated by platelet L-EVs (-20 %)
Spectre et al. Platelets 2019 58		EU (Sweden)	T2DM (n= 9) and T1DM (n= 11) patients Male:female= 8:1 (T2DM), 5:4(T1DM) Age= 55 (40-72, T1DM), 68 (60-76, T2DM) BMI= 23.5 (T1DM), 30 (T2DM)	A standardized meal (620 kcal: 30 % fat, 54 % CHO, 16 % protein)	Annexin V ⁺ L-EVs: <ul style="list-style-type: none"> ▪ CD41⁺ ± CD62P⁺ ± CD142⁺ platelet L-EVs ▪ CD144⁺ or CD62e⁺ endothelial L-EVs ▪ CD14⁺ monocyte L-EVs ▪ CD142(TF)⁺ L-EVs from Platelet-free plasma (13,000 g) by Flow cytometry (Gallios, BD)	<ul style="list-style-type: none"> • ↑ annexin V⁺ L-EVs in T2DM and T1DM patients at 90min after meal (+40 %) • ↑ thrombin generation by postprandial L-EVs compared to pre-meal L-EVs

Publication	Study design	Country	Participants	Intervention	Investigated EVs	Main results
Strohacker et al. Appl Physiol Nutr Metab 2012 59	Randomized Controlled	NA (US)	Healthy volunteers (n=8) Male:Female= 4:4 Age= 21 ± 3 BMI= 23.1 ± 3.92	Standardized test meal (1,070 kcal: 58 % from fat, 30 % CHO, 9 % proteins) ± prior endurance exercise or rest	CD31 ⁺ /CD42b ⁻ endothelial L-EVs From platelet-poor plasma (1,500 g) By flow cytometry (EasyCyte 6HT-2L, EMD Millipore)	<ul style="list-style-type: none"> • Test meal: ↑ postprandial (1h: +50 %) CD31⁺/CD42b⁻ endothelial L-EVs • Prior exercise blocks the postprandial ↑ of CD31⁺/CD42b⁻ endothelial L-EVs

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117 **Table 2 (To be continued)**

Publication	Study design	Country	Participants	Intervention	Investigated EVs	Main results
Sustar et al. Lipids in Health & Disease 2011 46	Cross-over	EU (Slovenia)	Healthy volunteers (n=21) Male:Female= 11:10 Age= 31 ± 10 (M), 29 ± 13 (F) BMI=not reported	2 days of food rich in cholesterol, fat and carbohydrates. On day 2, a breakfast consisting of two eggs, bread and a dairy product (7 a.m.) and small meal consisting of a dairy product (10 a.m.)	CD31 ⁺ and CD42b ⁻ endothelial L-EVs CD31 ⁺ and CD42b ⁺ platelet L-EVs CD235 ⁺ erythrocyte L-EVs From plasma L-EVs (17,570 g pellet) By flow cytometry (Altra, Beckman)	<ul style="list-style-type: none"> • postprandial total L-EVs (2 h: +52 %) • ∅ postprandial redistribution of L-EVs with respect to origin
Sutherland et al. Metabol Clin Exp 2010 60	Randomized Cross-over Single blinded	South Pacific (New Zealand)	Healthy volunteers (n= 22) Male:Female= 13:9 Age= 40 ± 13 BMI= 25.3 ± 3.7	n-6 PUFAs-rich meals containing unheat sunflower oil (USO) or heated SO (HSO) (510 Kcal: 72 % from Fat (<i>mainly</i> PUFAs), 25 % CHO, 2 % Proteins); C= saturated fat-rich meal containing cream (CR) (527 Kcal: 70 % from Fat (<i>mainly</i> SAFAs and MUFAs), 27 % CHO, 4 % Proteins)	CD144 ⁺ CD62b ⁻ endothelial L-EVs From platelet-poor plasma (6,000 g) by flow cytometry (FACSCalibur, BD)	↑ postprandial (3 h: +20 %) CD144 ⁺ endothelial L-EVs after sunflower oil enriched meals but not after CR meal
Tamburrelli et al. Thrombosis & Haemostasis 2012 61		EU (Italia)	Subjects at different degree of CV risk (n= 61) Male:Female= 40:21 Age= 48.6 ± 13.1 BMI= 26.7 ± 3.94	A standardized fatty meal (675 kcal: 69 % from fat, 15 % CHO, 15 % proteins)	CD42b ⁺ L-EVs By flow cytometry (EPICS LX Flow, Beckman)	↑ postprandial (2 h: +150 %) CD42b ⁺ L-EVs in men, not in women
Tushuizen et al. Diabetes Care 2007 47	Not reported	EU (The Netherlands)	Caucasian men with T2DM (n= 15, T2DM) or healthy (n= 12, H; aged-matched) Age= 55 ± 2 (T2DM) BMI=not reported	Three consecutive isocaloric meals (breakfast, lunch and dinner; 900 kcal: 50 % from fat, 33 % CHO, 15 % protein)	AnnexinV ⁺ CD144 ⁺ endothelial L-EVs From plasma By flow cytometry (FACScan, BD)	<ul style="list-style-type: none"> • At baseline, ↑ CD144⁺ endothelial L-EVs in T2DM (+130 %) • 4h after the last meal, ↑ CD144⁺ endothelial L-EVs in T2DM

Publication	Study design	Country	Participants	Intervention	Investigated EVs	Main results
						compared to healthy volunteers (+260 %)
Tushuizen et al. J Thrombosis and Haemostasis 2006 48	Randomized Cross-over	EU (The Netherlands)	Healthy men (n= 17) Age= 25.4 ± 3 BMI= 23.6 ± 1.8	I= Two consecutive standardized meals (900 kcal: 56 % from fat, 25 % CHO, 13 % protein) as breakfast and 4h later as lunch C= fasting	<ul style="list-style-type: none"> CD61 for platelet L-EVs CD62E, CD106 and CD144 for endothelial L-EVs CD4, CD8 and CD14 for leukocyte L-EVs From plasma By flow cytometry (FACScan, BD)	I= ↑ total L-EVs compared to C (+100 % over the 8 h of the study) I= ↓ FMD

118 **Table 2 (To be continued)**

Publication	Study design	Country	Participants	Intervention	Investigated EVs	Main results
Tushuizen et al. Thrombosis Res 2012 62	Randomized Cross-over	EU (The Netherlands)	Healthy men (n= 12) Age= 26.0 ± 0.9 BMI= 23.9 ± 0.4	I=Two consecutive standardized meals (900 kcal: 56 % from fat, 25 % CHO, 13 % protein) as breakfast and 4h later as lunch C= fasting	AnnexinV ⁺ associated with <ul style="list-style-type: none"> CD61 for Platelet L-EVs CD62E, CD106 and CD144 for endothelial L-EVs CD4, CD8 and CD14 for leukocyte L-EVs from plasma L-EVs (17,590 g pellet) By flow cytometry (FACScan, BD)	<ul style="list-style-type: none"> Over the postprandial period, ↑ erythrocyte-derived L-EVs (+220 %) After meals, ↑ phosphatidylcholine but not of phosphatidylserine in L-EVs compared to fasting

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122 Abbreviations

123 C: control diet, I: nutritional intervention, BMI: Body Mass Index, CV risk: Cardiovascular risk, T1/2DM: Type 1/2 Diabetes Mellitus, NA: North America, EU:

124 Europe, US: United States, CHO: carbohydrate, PUFAs: Poly Unsaturated Fatty Acid, EPA: EicosaPentaenoic Acid, DHA: DocosaHexaenoic Acid, NGT: Normo

125 Glucose Tolerance

2. The postprandial variation of circulating L-EVs

The response of L-EVs to the acute intake of different test meals (providing high dose of glucose, of saturated fat or enriched in polyunsaturated fatty acids) has been investigated during the postprandial period (Table 2)^{46-48, 51-62}. The studies reported on the level of L-EVs assessed mainly 2-3 hours after the intake of the test meal in comparison to fasting conditions or to the consumption of a control meal. In healthy young volunteers, the consumption of a meal supplying 19-70 % energy from fat (mainly saturated fat) increased significantly the level of circulating L-EVs at 2 hours postprandially from 20 to 160 %^{48, 51, 53, 54, 59, 60, 62}. Several subtypes of circulating L-EVs increased in response to fat intake: the endothelial L-EVs identified as CD31⁺-CD42b⁻ (+50-160 %) ^{53, 54, 59}, annexin V⁺-CD31⁺-CD42b⁻ (+30%) ⁵¹, CD144⁺ (+ 20 %) ⁶⁰ and CD144⁺-CD62b⁻ (+ 100-130 %) ^{47, 48} EVs, and the platelet-derived L-EVs characterized as annexin V⁺-CD31⁺-CD42b⁺ ⁵¹ and CD41⁺-CD61⁺ ⁵⁶ EVs. Studies from Tushuizen et al. ⁶², Sustar et al. ⁴⁶ and Spectre et al. ⁵⁸ also showed that all annexin V⁺ L-EVs increased postprandially after a high fat meal. One study did not observe a variation of L-EV levels after a high fat meal while they used CD31-CD42b-CD62 markers to identify the endothelial L-EVs ⁵⁵. An acute increase of L-EV levels during the postprandial period was also observed in diabetic patients ⁵⁸, in individuals with cardiovascular risk ⁶¹ and in atherosclerotic patients ⁵⁶, all of them presenting already a higher fasting level of L-EVs when compared to healthy volunteers. It is noteworthy that the postprandial increase of L-EV levels had similar magnitude in individuals at CVD risk than in healthy volunteers ⁵⁶. Furthermore, based on the studies from Table 2, no correlation was found between the percentage of dietary fat and the magnitude of L-EV variation. Other nutrients provided by food such as carbohydrates (CHO) or proteins may affect the postprandial level of L-EVs. To our knowledge, no study has so far investigated the impact of protein intake on the postprandial EVs. Regarding CHO, *in vitro* studies showed that a high concentration of glucose increased the secretion of L-EVs from endothelial cells ^{41, 63}, leucocytes ^{64, 65}, trophoblasts ⁴⁰ or mesangial cells ⁶⁶. In obese volunteers, Eichner et al. ⁵² reported that an oral glucose tolerance test, providing 75 g of glucose and resulting in a 136 mg/dL glycaemia at 2 hours, reduced the level of circulating L-EVs characterized in PFP. In addition, the postprandial level of CD31⁺-CD42b⁻ endothelial L-EVs increased from 30 to 160 %, 2

hours after the intake of a test meal providing 50-60 % of fat and 30-34 % of CHO (mainly simple sugars)^{51, 54}. Moreover, the study performed by Ferreira et al.⁵³ has demonstrated that a fat-free meal was not associated to an increased level of postprandial L-EVs compared to an isocaloric meal providing 19 % fat. Altogether, these data suggest that the percentage of CHO in the meal is not a determinant in the postprandial variation of L-EVs contrary to the fat content.

Diet may also provide some micronutrients (n-3 PUFAs) or microconstituents (polyphenols) that seem able to counteract the increase of postprandial EVs. The consumption of red wine, providing high amount of polyphenols, alleviated by 83 % the high-fat diet (HFD)-induced L-EVs⁵¹. Phang et al.⁵⁷ reported that 24 hours after an acute intake of 1 g of n-3 PUFAs, the level of plasma annexin V⁺-CD41a⁺ EVs was unchanged compared to a control meal. However, the intake of n-3 PUFAs reduced the capacity of these platelet-derived L-EVs to induce thrombin formation. Spectre et al.⁵⁸ reported that the postprandial annexin V⁺ L-EVs induced a higher thrombin generation than pre-meal annexin V⁺ L-EVs. Studies aiming at deciphering the biological function of postprandial EVs are scarce. Their functional activities could be predicted *in silico* by an enrichment-based bioinformatics analysis of the proteome and transcriptome of EVs, however the use of this approach is limited by the lack of data on protein content and nucleic acid content of the postprandial EVs.

To conclude, in so far as a meal provides saturated fats, its acute consumption is associated to a rapid rise of plasma L-EVs. In the absence of detailed pharmacokinetic data all over the postprandial phase, the transient character of the postprandial phase cannot be asserted to the EV rise. However, one can speculate that postprandial EVs temporary rise up as the clearance of endothelial- and platelet-derived L-EVs in the human circulation has been demonstrated to vary from 1 to 6 hours^{67, 68}. Lastly, some dietary constituents such as dietary polyphenols and n-3 PUFAs modulate positively the biology of EVs during the postprandial phase, that is, either by preventing the increase of EV levels or attenuating their bioactivity.

176 **Table 3: Human intervention studies reporting the effects of (sub)chronic dietary intake on circulating EVs.** C: control diet; I: nutritional intervention.

Publication	Study design	Country	Participants	Dietary intervention	Duration (weeks)	Investigated EVs	Main results
Del Turco et al. Haematologica 2008 45	Randomized Controlled	EU (Denmarks)	Post-myocardial infraction patients (n= 21, I ; n= 21, C) Male:Female= 18:3 Age= 65 ± 5 (I), 62 ± 8 (C) BMI= not reported	I= 5.2g total n-3 FA (4.3 g EPA+DHA) C= Olive Oil	12	All AnnexinV ⁺ / TF ⁺ L-EVs ▪ CD61 ⁺ platelet L-EVs ▪ CD14 ⁺ monocyte L-EVs ▪ CD62E ⁺ endothelial L-EVs from 17,500 g pelleted plasma L-EVs	I=↓ CD61 ⁺ L-EVs, ↓ CD14 ⁺ L-EVs, ∅ CD62e ⁺ endothelial L-EVs, ∅ TF ⁺ L-EVs I= ↓ thrombin generation by L-EVs C=∅ L-EVs At baseline, ↑ total L-EVs in patients compared to healthy matched volunteers (n= 10)
Durrer et al. Nutrients 2019 69	Randomized Short-term Controller Cross-over	EU (UK)	Healthy men (n= 9) Age= 21 ± 3 BMI= 23.2 ± 2	○ A 7-day intervention on low-carbohydrate/high-fat diet (2417 ± 267 kcal: 71 % fat, 11 % CHO, 18 % protein) after 2 weeks of typical diet (2584 ± 456 kcal: 37 % fat, 46 % CHO, 17 % protein) ○ 75 g oral glucose tolerance test (oGTT) performed before and after nutritional interventional	1	▪ CD31 ⁺ /CD42b ⁻ endothelial L-EVs ▪ CD62E ⁺ endothelial L-EVs From platelet-poor plasma (1,500 g) By flow cytometry (LSRII, BD)	At fasting, no difference in L-EVs levels from LCHFD group compared to typical diet group ↑ post oGTT CD31 ⁺ /CD42b ⁻ and CD62E ⁺ endothelial L-EVs (1 h) in LCHFD group LCHFD: ↓ FMD
Eitan et al. Aging Cell 2017 70	Randomized Controlled	NA (US)	Prostate cancer patients (n= 38) Age= 59.26 ± 7.5 BMI= 30.45 ± 5.8	I= protein restriction diet (2856 ± 198 kcal: 31 % fat, 59 % CHO, 8 % protein) C= regular diet (2367 ± 444 Kcal: 37 % fat, 45 % CHO, 17 % protein)	4	L1CAM immunoprecipitated EVs from Precipitated total plasma EVs by NTA	I: ∅ EV size distribution, ∅ total EV concentration I: ↑ Leptin Receptor ⁺ /L1CAM ⁺ neuronal EVs, ↑ pSer312 IRS1 ⁺ /L1CAM ⁺ neuronal EVs
Francois et al. Am J Physiol Heart Circ Physiol 2018 71	Randomized Cross-over Controlled	NA (Canada)	T2DM volunteers (n= 16) Male:Female= 8:8 Age= 64 ± 8 BMI= 34.0± 8	2 isocaloric meals (500 kcal) ○ Low-fat control diet (CON: 20 % fat, 55 % CHO, 25 % protein) ○ low-carbohydrate high-fat diet (LC: 65 % Fat, 10 % CHO, 25 % protein) ○ LC with 15-min post-meal walks (LC-EX)	4 days	▪ CD62E ⁺ endothelial L-EVs ▪ CD62P ⁺ platelet L-EVs From platelet-free plasma (13,000 g) By flow cytometry (FACSAria III, BD)	LC : ↓ CD62E ⁺ endothelial L-EVs (- 50 %) ; ∅ CD62P ⁺ L-EVs LC-EX : ∅ CD62E ⁺ endothelial L-EVs ; ↓ CD62P ⁺ L-EVs (- 44 %) CON : ↑ CD62E ⁺ endothelial L-EVs (+ 56 %) ; ∅ CD62P ⁺ L-EVs

Publication	Study design	Country	Participants	Dietary intervention	Duration (weeks)	Investigated EVs	Main results
Gröne et al. JAF 2019 ⁷²	Randomized Double-blinded Controlled	EU (Germany)	Healthy men (n= 39) Age= 26 ± 3 (Young Group, n= 20), 60 ± 8 (Elderly Group, n= 19) BMI= 24.1 ± 2.3 (Young Group), 26.5 ± 3.2 (Elderly Group)	I= cocoa flavanol drink (CF, providing 450 mg of total flavanol, twice daily) C= nutrients-matched CF free drink	2	CD62e ⁺ endothelial L-EVs CD144 ⁺ endothelial L-EVs CD31 ⁺ /CD41 ⁻ endothelial L-EVs from Platelet-free plasma (10,000 g) by Flow cytometry (VERSE, BD)	I: ↓CD31 ⁺ /CD41 ⁻ endothelial L-EVs (Y: -52 %; E: -57 %), ↓CD144 ⁺ endothelial L-EVs (Y: -23 %; E: -21 %), ↓CD62e ⁺ endothelial L-EVs (Y: -35 %; E: -24 %) in Young and Elderly C: ↑CD31 ⁺ /CD41 ⁻ endothelial L-EVs (+52 %), ↑CD144 ⁺ endothelial L-EVs (+35 %), ↑CD62e ⁺ endothelial L-EVs (+57 %) in elderly vs young
Horn et al. BJN 2014 ⁷³	Randomized Double-blinded Cross-over	EU (Germany)	CAD patients (n= 16) Male:Female= 13:3 Age= 64 ± 3 BMI= 28.8 ± 1.8	I= high-cocoa flavanol drink (providing 375 mg of total flavanol) C= Low coca-flavanol drink (9 mg)	4	CD41 ⁺ platelet-derived L-EVs CD144 ⁺ endothelial L-EVs CD31 ⁺ /CD41 ⁻ endothelial L-EVs from Platelet-free plasma (10,000 g) by Flow cytometry (Canto II, BD)	I: ↓CD144 ⁺ endothelial L-EVs (-22 %), ↓CD31 ⁺ /CD41 ⁻ endothelial L-EVs (-39 %), Ø CD41 ⁺ PL-EVs in CAD patients C: ↑ CD144 ⁺ (+ 490 %) and CD31 ⁺ /CD41 ⁻ (+ 30 %) endothelial L-EVs in CAD patients vs age-matched healthy volunteers
Marin et al. AJCN 2011 ⁷⁴	Randomised Cross-over	EU (Spain)	Healthy elderly volunteers (n= 20) Male:female=10:10 Age= 67.1 ± 4.52 BMI= 31.9 ± 5.50	3 diets providing 1910 Kcal/day - Mediterranean Diet (Met Diet): 38 % fat (24 % MUFAs, <10 % SFA), 47 % CHO, 15 % protein - SFA-rich diet: 38 % fat (12 % MUFAs, 22 % SFAs, 4 % PUFAs), 47 % CHO, 15 % protein - Low-fat high-carbohydrate diet enriched in n-3 PUFAs (CHO-ALA): <30 % fat (<12 % SFAs, 12 % MUFAs, 8 % PUFAs), 55 % CHO, 15 % protein	4	AnnexinV ⁺ CD31 ⁺ endothelial L-EVs CD144 ⁺ /CD62e ⁺ activated endothelial L-EVs from Platelet-free plasma (13,000 g) by Flow cytometry (FC500, Beckman)	I: Met Diet and CHO-ALA diet ↓ total (-60 %; -33 %), CD144 ⁺ /CD62e ⁺ (-76 %; -34 %) and annexinV ⁺ /CD31 ⁺ (-70 %; -25 %) endothelial L-EVs vs SFA diet I: Met Diet ↓ total (-40 %), CD144 ⁺ /CD62e ⁺ (-63 %) and annexinV ⁺ /CD31 ⁺ (-60 %) endothelial L-EVs vs CHO-ALA diet

Publication	Study design	Country	Participants	Dietary intervention	Duration (weeks)	Investigated EVs	Main results
McFarlin et al. JNB 2015 ⁷⁵	Randomized Controlled Cross-over	NA (US)	Healthy volunteers with normal weight (n= 10, N), over-weight (n= 7, O) or obesity (n=7, Ob) Age= 21 ± 2 (N), 22 ± 3 (O), 22 ± 3 (Ob) BMI= 21.6 ± 1.9 (N), 27.0 ± 1.4 (O), 34.9 ± 3.0 (Ob)	I= natural cocoa bars (supplying 13.6 mg catechin, 48 mg epicatechin, 640 mg total proanthocyanidins) C= placebo bars	4	CD144 ⁺ /CD42a ⁻ /CD45 ⁻ endothelial L-EVs from Platelet-free plasma (15,000 g) by Flow cytometry (FC500, Beckman)	I: ↓CD144 ⁺ /CD42a ⁻ /CD45 ⁻ L-EVs concentration in O (-13 %) and Ob (-31 %). C: ↑baseline CD144 ⁺ /CD42a ⁻ /CD45 ⁻ L-EVs concentration with ↑BMI (+21 % compared to O; +53 % compared to N)
Morel et al. Int J Obesity 2011 ⁷⁶	Cross-over	EU (France)	Obese women (n= 24) Age= 39 ± 10 BMI= 35 ± 4	I= diet restriction (600 kcal/day: 15 % Fat, 30 % CHO, 55 % Protein) for 1 month followed by a regular diet (1200 kcal/day: 10 % Fat, 60 % CHO, 30 % protein) for 1 month and a progressive normal intake (1 month)	12	AnnexinV ⁺ L-EVs CD11a ⁺ leukocyte L-EVs CD4 ⁺ lymphocyte L-EVs CD31 ⁺ CD41 ⁻ endothelial L-EVs CD41 ⁺ platelet L-EVs CD235 ⁺ erythrocyte L-EVs from Platelet-free plasma (13,000 g) by Flow cytometry (Gallios, BD)	1 month-diet restriction ↑ total AnnexinV ⁺ L-EVs (including CD41 ⁺ L-EVs, CD235 ⁺ L-EVs) I= ↓ CD4 ⁺ (-20 %), CD11a ⁺ (-28 %) and procoagulant-platelet derived L-EVs (-43 %)
Murakami et al. Thrombosis Research 2007 ⁷⁷	Randomized Controlled	Asia (Japan)	Obese (n= 49, O) and healthy non-obese (N= 37, N) volunteers Male:Female= 21:16 (N), 24/25 (O) Age= 49.2 ± 1.8 (N), 50.6 ± 1.4 (O) BMI= 22.8 ± 0.2 (N), 27.4 ± 0.3 (O)	I= energy restricted diet (1200 kcal/day for women; 1680 kcal/day for men) ± aerobic exercise 3 days/weeks (60 min/session)	12	CD41 ⁺ platelet L-EVs From platelet-rich plasma (200 g) By flow cytometry (FACScan, BD)	At baseline: ↑ CD41 ⁺ L-EVs in O (+97 %) I: energy restricted diet ↓ CD41 ⁺ L-EVs (-30 %)
Myette-Côté et al. Physiol 2018 ⁷⁸	Randomized Short-term Controller Cross-over	EU (UK)	T2DM volunteers (n= 11) Male:Female= 4:7 Age= 64 ± 8 BMI= 34.0 ± 8.0	○ Low-fat low-glycemic index diet (GL: 20 % fat, 55 % CHO, 25 % protein) ○ Low-carbohydrate high-fat diet (LC: 65 % Fat, 10 % CHO, 25 % protein) ○ LC with 15-min post-meal walks (LC-EX)	4days	CD14 ⁺ monocytes L-EVs CD45 ⁺ leukocyte L-EVs From platelet-free plasma (13,000 g) By flow cytometry (FACSaria III, BD)	GL and LC ↓ both plasma CD14 ⁺ L-EVs (-70 %) and CD45 ⁺ LL-EVs (-63 %; 16 %; tendency)

181 Abbreviations: oGTT: oral Glucose Tolerance Test, MUFA: Mono Unsaturated Fatty Acid, SFA: saturated fatty acids.

Publication	Study design	Country	Participants	Dietary intervention	Duration (weeks)	Investigated EVs	Main results
Phang et al. Nutr CVD 2016 ⁷⁹	Randomized Controlled Double-blinded Cross-over	South Pacific (Australia)	Healthy volunteers (n=94) Male:Female= 43:51 Age= 39.6 ± 1.7 BMI= 24.6 ± 3.7	I= EPA rich oil (500:100 mg EPA:DHA) or DHA rich oil (100:500 mg EPA:DHA) C= sunola oil	4	AnnexinV ⁺ /CD41 ⁺ /CD62 ⁺ platelet L-EVs From platelet-free plasma (3,000 g) by flow cytometry (Canto, BD)	At baseline, ∅ effect gender on the number of endothelial L-EVs I= ∅ AnnexinV ⁺ /CD41 ⁺ /CD62 ⁺ L-EVs
Wekesa et al. Appl. Nutr 2016 ⁸⁰	Randomized Cross-over	EU (Ireland)	Overweight women (n=28) Age= 48.4 ± 0.6 BMI= 28.1 ± 2.7	I= low-carbohydrate diet (1228 Kcal: 48 % fat, 29 % CHO, 23 % protein) C=normal diet (1626 Kcal: 38 % fat, 44 % CHO, 18 % protein)	24	CD31 ⁺ /CD41 ⁻ Endothelial L-EVs CD144 ⁺ or CD146 ⁺ or CD105 ⁺ Endothelial L-EVs (namely endothelial L-EVs) from Platelet-poor plasma (13,000 g) by Flow cytometry (FC500, Beckman)	I= ↓ CD31 ⁺ /CD41 ⁻ endothelial L-EVs (-30 %), ∅ endothelial L-EVs
Wu et al. AJCN 2014 ⁸¹	Randomized Controlled Double-blinded Cross-over	EU (UK)	Moderate CVD risk volunteers (n= 84) Male:Female= 29:55 Age= 45.8-49.3 BMI= 25.6-26.6	I= fish-oil supplementation providing 1.5g LC n-3 PUFAs C=corn-oil supplementation	8	CD31 ⁺ /CD42b ⁻ endothelial L-EVs CD31 ⁺ /CD42b ⁺ PL-EVs from platelet-poor plasma (1,000 g) by flow cytometry (FACSCalibur, BD)	I= ↓ CD31 ⁺ /CD42b ⁻ endothelial L-EVs (-10 %)
Zhang et al. MNFR 2014 ⁸²	Randomized Cross-over	EU (UK)	T2DM patients (n= 22) Mixed gender Age from 40 to 75 years BMI= not reported	○ oat-rich habitual diet (131 g/day, OAT) ○ standard dietary advice (SDA) ○ habitual diet (HAB) Challenge meal (720 Kcal: 33 % fat, 53 % CHO, 14 % protein)	8	C61 ⁺ ± Fibrogen ⁺ or TF (CD142) ⁺ or CD62P ⁺ platelet L-EVs CD45 ⁺ ± CD14 ⁺ ± CD11b ⁺ leukocyte L-EVs from platelet-poor plasma (2,000 g) by flow cytometry (not detailed)	• OAT: ↓ Fibrogen ⁺ (-44 %) and TF ⁺ (-43 %) platelet L-EVs, CD11b ⁺ (-74 %) ML-EVs compared to HAB • SDA: ↓ Fibrogen ⁺ L-EVs compared to HAB (-47 %) During the postprandial period after challenge meal: • Transient ↓ of leukocyte-derived L-EVs in all groups • ↓ of monocyte L-EVs and CD11b ⁺ L-EVs in OAT and SDA; ↑ in HAB
Weech et al. An. J. Clin. Nutr. 2018 ⁸³	Randomized, controlled, single-blind	EU (UK)	Men and Women with moderate CVD risk (n=190) Male:Female=82:108 Age=45 ± 1 BMI= 26.3 ± 0.5	I=replacement of 8% SFAs with MUFAs or n-6 PUFAs for 16 weeks		▪ CD31 ⁺ CD42b ⁻ for endothelial L-EVs ▪ CD31 ⁺ CD42b ⁺ for platelets L-EVs From platelet-poor plasma by flow cytometry (FACSCalibur, BD)	• Replacement by MUFAs: ↓ endothelial L-EVs (-47.3 %) and platelet L-EVs (-36.8 %)° • Replacement by n-6 PUFAs: ↓ endothelial L-EVs (-44.9 %) and platelet L-EVs (-39.1 %)° • SFA diet: ↑ endothelial L-EVs (+

3. Chronic dietary interventions modulate plasma L-EVs at fasting

Acute interventions have revealed that dietary intakes can drive the release of L-EVs during the postprandial period. Outside of this period (e.g., at fasting state), there still have detectable L-EVs in the circulation and have been of interest as reliable biomarkers for diagnosis and prognosis since a proportional increased level has been observed in several conditions, including ageing^{72, 84}, physical activity⁸⁵, metabolic disorders such as obesity^{12, 86}, type 2 diabetes^{12, 13} and metabolic syndrome⁸⁷, and CVD⁸⁸. Based on the established relationship between diet and health²³, some research has been done to decipher the effects of (sub)chronic dietary interventions on plasma remnant EVs in different categories of population (e.g., healthy, young or normo-weighted persons, at risk, patients) (Table 3)^{45, 69-83}. The efficiency of these interventions, lasting from 4 days to 24 weeks, has been assessed by comparing the plasma levels of L-EVs between the beginning (baseline) and the end of the study period or by comparing levels between study groups, depending on their design.

Two studies reported that diet restriction aiming at reducing the dietary energy intake of volunteers with a BMI over 25 kg/m² significantly reduced the level of circulating L-EVs. In obese women, a 600 kcal/day diet restriction for one month followed by two months of regular (1200 kcal/day) and normal diets results in lowering plasma levels of lymphocyte-derived (CD4⁺, -20 %), leukocyte-derived (CD11a⁺, -28 %) and procoagulant platelet-derived L-EVs (-43 %) ⁷⁶. In healthy middle-age volunteers, the increase of resident platelet-derived L-EVs (CD41⁺) associated to obesity can be attenuated by 30 % after 12 weeks under an energy-restricted diet⁷⁷. Another randomized controlled trial showed that a low protein diet (8%) administrated to prostate cancer patients did not affect the level of circulating cancer-derived L-EVs identified using L1CAM markers, but enriched some of the L-EV surface markers (Leptin Receptor and phospho-Ser-IRSII), supporting the effectiveness of the dietary intervention to affect level of plasma L-EVs⁷⁰.

Dietary CHO, including sugars, starches, celluloses and gums serve as major energy sources of human diet. The physiological effects of dietary CHO are highly dependent on the rate and extent of digestion and absorption in the small intestine and fermentation in the large intestine by the gut

microbiota, these latter interactions being known to promote health. Thereby, the modulation of the CHO fraction could be useful to reach a healthier quality. Wekesa et al.⁸⁰ reported that a low-CHO diet decreases the level of circulating CD31⁺/CD41⁻ endothelial L-EVs (30 %) in middle-aged over-weight healthy women. However, low-CHO content is often offset by an increased fat supply to maintain the energy content of the diet, that is why several studies have investigated the impact of a low CHO-high fat diet. Durrer et al.⁶⁹ did not observe any variation of CD31⁺/CD42b⁺ and CD62⁺ endothelial L-EVs in fasting plasma of young healthy volunteers after a 7 days- intervention on a low CHO-high fat diet. However, higher levels of plasma L-EVs were observed after a metabolic challenge consisting in an oral glucose tolerance test only in volunteers that consumed the low CHO-high fat diet. In type 2 diabetes volunteers, a low CHO-high fat diet reduced the level of plasma CD62⁺ endothelial L-EVs (-45 %) ⁷¹ and leukocyte-derived L-EVs (-63 % of CD45⁺ L-EVs, -70 % of CD14⁺ L-EVs) ⁷⁸. In another study, the consumption for 8 weeks of an oat-rich diet (providing complex CHO, extensively metabolized by the gut microbiota) has led to a lower level of fasting circulating fibrogen⁺-, TF⁺-CD61⁺ platelet L-EVs (around -40 %) and of leukocytes-derived L-EVs after a challenge test meal in type 2 diabetes (T2DM) patients ⁸². Taken together, these results highlight that dietary CHO content affects the basal level of circulating L-EVs. Most of the aforementioned studies did not specify the CHO quality used while a relationship between CHO quality and health have been demonstrated ⁸⁹, especially regarding CHO with a low glycaemic index and those poorly absorbable but highly metabolized by the gut microbiota. The nutritional interventions presented in this review provide low amount in total CHO ^{69, 71, 80} or high in low glycaemic index CHO ^{78, 82} and have been associated with a decrease in circulating L-EVs.

An intervention based on a Mediterranean diet, one of those peculiarity is to be rich in n-3 PUFAS (and in a diversity of plant food bioactives), reduce the level of circulating endothelial L-EVs identified as annexin V⁺-CD31⁺ and CD144⁺-CD62e⁺ vesicles in elderly volunteers ⁷⁴. In the same study, the authors have also demonstrated that a low-fat – high CHO diet enriched in n-3 PUFAs (8 %) modulated similarly the level of circulating endothelial L-EVs. In another randomized controlled trial, some subtypes of circulating L-EVs (namely CD61⁺ platelet-derived and CD14⁺ monocyte-derived L-EVs) present in high

amounts in post-myocardial infarction patients were reduced by a 12 weeks- supplementation with n-3 PUFA ⁴⁵. Likewise, a fish-oil supplementation providing long chain n-3 PUFAs and saturated fatty acids replacement by MUFA or n-6 PUFAs reduced the circulating level of CD31⁺/CD42b⁻ endothelial L-EVs and/or platelet L-EVs in volunteers with moderate risk of CVD ^{81, 83}. In contrast, in healthy middle-age volunteers, the 4-weeks diet enrichment in n-3 PUFAs failed to modulate the level of circulating annexin V⁺-CD41⁺-CD62⁺ platelet L-EVs ⁷⁹.

Beyond their potential contribution to the observed modulatory effects of a Mediterranean Diet on circulating L-EVs ⁷⁴, the efficiency of polyphenols *per se* to modulate circulating L-EVs has also been investigated. Cocoa polyphenols have been of interest due to their well-known protective effects improving CV health ⁹⁰. Thus, McFarlin et al. ⁷⁵ reported that a 4-weeks dietary supplementation with cocoa bars supplying flavanols and proanthocyanins (700 mg/d in total), reduced by 13% the concentration of endothelial L-EVs (identified as CD144⁺/CD42a⁻/CD45⁻) in over-weight young people. Similarly, the 52% higher level of CD31⁺/CD41⁻ endothelial L-EVs observed in healthy elderly men compared to young men can be reduced by 50% after only 2 weeks of nutritional intervention supplying a cocoa flavanol rich drink twice a day (450 mg/daily dose) ⁷². Lastly, the consumption of this cocoa flavanol rich drink for 4 weeks has been demonstrated to reduce significantly the levels of CD144⁺ endothelial L-EVs (-22 %), CD31⁺/CD41⁻ endothelial L-EVs (-39 %) but not of CD41⁺ L-EVs in CAD patients ⁷³.

In conclusion, the analysis of these (sub)chronic intervention studies shows that dietary habits can affect the level of circulating L-EVs. Interventions aiming at providing a healthier diet, such as at low-glycaemic index or rich in n-3 PUFAs and plant food bioactives (e.g. polyphenols) appear efficient to reduce the level of circulating endothelial L-EVs and platelet-derived L-EVs in healthy volunteers or in patients with cardiometabolic disorders.

4. Dietary modulation of EVs: what potential underlying mechanisms of action?

The release of S-EVs resulting the fusion of the MVB with the plasma membrane and the budding of L-EVs are mechanisms both highly regulated. They involved a wide range of factors which regulate (i)

deformability and bending of the membrane inside MVB or at the plasma membrane (i.e. the endosomal sorting complexes required for transport or ESCRT; the generation of ceramide; the tetraspanins), membrane lipid composition, intracellular trafficking (e.g. Rab GTPases and the soluble N-ethylmaleimide-sensitive factor activating protein receptors or SNAREs) and (ii) organization of the actomyosin cytoskeleton (Rho GTPases)^{8, 91-96}. In the perspective that, as suggested by the present review of available data, the diet can impact EVs both in fasting and postprandial conditions, it would be interesting to understand how some macro-/micro-nutrients (glucose, amino acids, triglycerides, and n-3 PUFAs) and microconstituents (such as polyphenols, as main plant food bioactives) act to trigger physiological pathways controlling the biogenesis and/or the release of EVs.

The postprandial period is characterized among other metabolic changes by a transient increase in plasma glucose concentration whose magnitude depends on glucose load and on physiological state. *In vitro* studies have demonstrated that a moderate elevation in glucose concentration (from 1g/L (5.5 mM) to 1.9 g/L (11 mM)) or higher (up to 9g/L (50 mM)) increases the release of L-EVs by endothelial cells^{39, 41, 63}, monocytes/leukocytes^{64, 65} or renal mesangial cells⁶⁶. However, Eichner et al.⁹⁷ observed that the level of plasma L-EVs (platelet-derived CD31⁺-CD41⁺ EVs, endothelial-derived CD31⁺-CD41⁻/CD105⁺ EVs) is lowered in obese adults with prediabetes at 2-h following administration of a single 75-g oral glucose load leading to 1.36 g/L plasma glucose. In contrast, an increased level of basal EVs has been reported in prediabetic individuals compared to individuals with normal glucose tolerance⁹⁸. Consistently, chronic dietary interventions showed a direct correlation between CHO diet content and the level of plasma L-EVs^{71, 78, 80}. This suggests that dietary glucose can drive L-EV release. The underlying molecular mechanisms supporting the action of plasma glucose on L-EV release have not been properly explored. High glucose can activate several signalling pathways including mitogen-activated protein kinases (MAPK) and Rho-GTPases (as Rho A-ROCK)⁹⁹⁻¹⁰¹ that have been reported to target the production of EVs^{102, 103}. The rise in glucose level also coincides with a rapid insulin secretion from pancreatic beta cells that exerts a hypoglycaemic action. To our knowledge, the effect of insulin on EV biogenesis has never been investigated. However, it is known that insulin regulates the intracellular vesicle trafficking machinery to

deliver the glucose transporter (GLUT4) to the cell surface and ensure glucose uptake. The insulin pathway engages small GTPases of RAB and ARF families (including the ADP-ribosylation factor 6, ARF6), tethering complexes and the vesicle fusion machinery (including the remodelling of the actin cytoskeleton and SNARE proteins)^{67, 104, 105}. Some of these molecular actors are also involved in the biogenesis and the release of L-EVs^{8, 91-96} suggesting that hyperinsulinemia may directly contribute to L-EV release.

The digestion of dietary proteins leads to a plasma increase of essential and branched-chain amino acids (BCAAs) from 40 to 120 min after protein intake¹⁰⁶⁻¹⁰⁸. The concomitant increase of aminoacidaemia and plasma EVs sustains a potential role of amino acids in the regulation of S-EV release. The main cellular amino acid sensors are the amino acid-sensitive kinase complex, mechanistic Target of Rapamycin Complex 1 (mTORC1) and the General Control Non-depressible Kinase 2 (GCN2)¹⁰⁹⁻¹¹¹. Briefly, under BCAA deficiency, the phosphorylated GCN2 contributes to the inhibition of mTORC1 and by consequence to the down regulation of protein synthesis. The low amino acids level is compensated by an enhance activity of autophagy or protein degradation mediated by the proteasome system to recycle damaged proteins back into BCAAs¹¹². Evidences have disclosed that a crosstalk between proteolytic activities (e.g. autophagy, proteasome and lysosome) and the release of S-EVs, with a balance to a reduced EV biogenesis under starvation conditions^{113, 114}. After a meal, the increase of aminoacidaemia and of insulin will restore the activity of mTORC1 contributing to alleviate the proteolytic activities and stimulate S-EV release^{115, 116}. mTORC1 activation can also be driven by the phosphatidic acid of EV layers that has been detected in EV-like nanoparticles¹¹⁷. Specifically, in endothelial cells, the activation of mTORC1 contributes through reactive oxygen species (ROS) to impairment of the endothelial function^{118, 119}, which one feature is the increased release of endothelial L-EVs⁷. In addition, the endothelial dysfunction induced by a high-fat diet during the postprandial period can be attenuated by a dietary supply in proteins (mainly proteins rich in arginine and cysteine)¹²⁰⁻¹²². Taken together, these observations support that dietary proteins can prevent EV release associated to postprandial cell dysfunctions. However, the lack of scientific evidence sustains the interest to explore the link between dietary BCAAs and the release of EVs.

Postprandial lipaemia is characterized by a rise in circulating free fatty acids (FFAs) and triglyceride-rich proteins (TRPs) that include intestine-derived chylomicrons and their remnants and liver-derived VLDLs¹²³. The postprandial lipaemia peak occurs at 2 hours after meal intake, kinetic that depends on dietary factors (e.g. diet composition, lipid quantity, food structure), intrinsic factors (e.g. gender, age, genetic background, health status) and life style factors (e.g. physical activity, smoking)^{124, 125}. The postprandial FFAs can directly drive the release of S- and L-EVs^{126, 127} acting on specific receptors such as the death receptor 5 (DR5)¹²⁷. With TRPs, they can also bind to other receptors such as the toll-like receptor 4 (TLR-4), contributing to induce a transient postprandial low-grade inflammation¹²⁸⁻¹³¹. In this way, postprandial lipaemia contributes to the development of a postprandial endothelial dysfunction^{128, 132} associated with an enhanced release of EVs. In addition, metabolic changes associated to the postprandial period, mainly postprandial glucose and lipids, participate to generate oxidative stress¹³³⁻¹³⁵ that sustains the postprandial development of low-grade inflammation state contributing to the release of EVs. Dietary n-3 PUFAs, in contrast to palmitic acids, participate lowering level of plasma EVs through a potent multi-level action that include their anti-inflammatory effect and their ability to modulate the mTOR pathway^{136, 137}.

Some dietary microconstituents such as polyphenols peak as metabolites in the plasma during the postprandial period¹³⁸. Dietary polyphenols have been reported to prevent the release of EVs but the underlying mechanism of action is unknown. Regarding their effects on endothelial-derived L-EVs, one can speculate that polyphenols act through their capacity at preserving endothelial function¹³⁹ and cell dynamics¹⁴⁰ to modulate the release of L-EVs. In addition, polyphenols have been demonstrated to counteract glucose-induced inhibition of autophagy in endothelial cells¹⁴¹ suggesting that they can restore cellular homeostasis and by consequence L-EV secretion¹⁴². Polyphenols display potent mTOR modulator through the attenuation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, that can lead the regulation of L-EV synthesis and release as previously shown¹⁴³.

Taken together, these sustain that diet-derived metabolites that circulate during the postprandial period can constitute modulators of EV secretion as summarized in Figure 1. Nevertheless, further

investigations are needed to decipher the underlying mechanisms of action. Furthermore, only the release of EVs has been considered here, while EV content that support EV bioactivity has never been investigated. Thus, another important question to address in future is how the diet can modulate the content of EVs, and by consequence their functions.

5. Food-derived EVs: can they affect human health?

EVs from exogenous dietary sources such as milk or vegetables can be also found in bloodstream and affect homeostasis of the host or improve pathophysiological conditions. This is the particular interest taking in consideration the ability of EVs to protect some molecules with short half-life from degradation. For instance, EV-like nanoparticles from grapefruits and milk resist to digestion products of the host such as gastric enzymes and intestinal pancreatic and bile extract solution. Furthermore, these nanoparticles do not modify serum levels of interferon gamma (IFN- γ) and the liver enzymes in mice suggesting the lack of deleterious effects on inflammation²³.

Interestingly, plant-derived EVs can display beneficial effects under pathological conditions due to the plant bioactive molecules that they carry, as recently reviewed^{43, 144, 145}. Indeed, it has been shown that grapefruit-derived nanovesicles can be used as immune modulators in the digestive tract during colitis in mice. Thus, through the direct action on intestinal macrophages, these vesicles inhibit the production of IL-1 β and TNF- α in these cells and allow the maintaining of intestinal macrophage homeostasis²³. In the same pathology, broccoli-derived nanoparticles decrease the weight loss, the infiltration of inflammatory cells in the mucosa and the pro-inflammatory cytokine production³⁰. Interestingly, broccoli-derived nanoparticles activate AMPK in dendritic cells leading to a dual effect associated to the prevention of dendritic cell activation and the induction of tolerant dendritic cells. Also, lemon-derived EVs display anti-tumor properties in several tumor cell lines by arresting cell proliferation of tumor cells *via* the induction of TRAIL-mediated cell death¹⁴⁶ and downregulating proteins implicated in the lipid metabolism pathway¹⁴⁷. Dietary plant-derived EVs had also effects on lipid metabolism as illustrated by the fact that ginger-derived EVs can protect against the accumulation of lipid droplets during alcohol-induced liver damage¹⁴⁷ and lemon EVs reduce LDL cholesterol in healthy volunteers¹⁴⁸.

Milk is another food source of EV-like nanoparticles that could modulate several functions of human milk-consumers triggering several host tissues¹⁴⁹. In human food, milk and its derived products can be pasteurized and/or fermented. Milk-derived EVs numbers are not affected by pasteurization compared to other method of sterilization^{150, 151}. Thus, pasteurized milk is a new source of large and small EVs for human consumers of western diet allowing the transfer of milk-derived microRNAs. These small EVs of pasteurized milk convey oncogenic micro RNAs (e.g., microRNA-21 and microRNA-148a) which both inhibit PTEN (Phosphatase and TENsin homolog) expression and drive mTORC1-mediated cancerogenesis¹⁵²⁻¹⁵⁴. In another study, the authors observed that bovine milk-EVs (orally administrated at 25 mg/kg) reduce primary tumor growth but accelerate metastasis in breast and pancreatic cancer mouse models¹⁵⁵. The oral administration of milk-derived S-EVs have been also demonstrated to attenuate the severity of colitis¹⁵⁶ and of arthritis¹⁵⁷. These beneficial effects have been partly associated to the ability of milk-derived S-EVs to reduce the expression of inflammatory cytokines (e.g. the monocyte chemo-attractant protein (MCP-1), inteleukin-6 and TNF- α) and to up-regulate the expression of anti-inflammatory mediators such as the transforming growth factor beta (TGF- β). In addition, milk-derived S-EVs convey miRNAs that can act as epigenetic regulators contributing to the effects of these EVs on biological functions¹⁵⁸. In addition, milk -derived S-EVs can drive some pathways such as the AMPK-mTORC1 activation^{152, 159}, that can result in the release of EVs from the host recipient cells.. Furthermore, due to their small size EV-like nanoparticles from foods can probably cross the epithelial barrier including the blood brain barrier¹⁶⁰. This hypothesis is supported by the loss of spatial learning and memory observed in mice fed a diet with bovine milk depleted in S-EVs for up to 20 weeks compared to mice consuming a complete diet¹⁶¹ and the detection in brain of fluorescent-labelled milk S-EVs administered to adult mice through oral gavage¹⁶².

Together, these data underline the strength of EV-like nanoparticles from foods to impact on biological functions and the interest to consider these EVs as relevant dietary contributors on human health.

6. Diet-induced EVs: friends or foes for human health?

It is widely demonstrated that EVs are important mediators of cell-to-cell communication. EV-mediated intercellular communication begins by the recognition and the capture of circulating EVs by target cells^{92, 163}, that is influenced by both EV surface markers and EV size. Information are transmitted from EVs to cells by acting at the cell membrane, with or without the delivery of their content. The mechanisms of uptake and cargo delivery into recipient cells are still incompletely understood. But evidences support that EVs drive phenotypic changes mostly by spreading the phenotypic features of donor cells to acceptor cells¹⁶⁴. Thus, the pending question that is raised in the present review focuses on the biological function(s) of diet-induced EVs. Knowledge on this topic is still scarce and is constrained by the heterogenicity of circulating diet-induced EVs. As previously mentioned, diet modulates EV release mainly from endothelial cells, leukocytes and platelets. In addition to this board of cell source, it has been reported that cells release various subpopulations of EVs with distinct size, protein cargo and miRNAs content^{36, 165-168}.

Spectre et al.⁵⁸, who reported an increased level of annexin V⁺ L-EV derived from endothelial cells, platelet and monocytes after a standard meal, have demonstrated that these EVs generate more thrombin than pre-meal EVs. In contrast, acute and chronic n-3 PUFA supplementation have been shown to lower plasma annexin V/TF⁺ L-EV levels and their thrombin generation capacity^{45, 57}. Endothelial-derived L-EVs carrying TF⁺ and platelet-derived L-EVs exposing at their surface phosphatidylserine are known to be procoagulant and to increase the risk of venous thrombo-embolism¹⁶⁹. Circulating L-EVs released in dyslipidaemia induced by a high-energy diet have been shown to contribute to inflammation and vascular damage by increasing the endothelial expression of intercellular adhesion molecule 1 (iCAM-1) and the production of reactive oxygen species and by reducing vascular relaxation of aortic rings²¹. These EVs also exert an antiatherogenic action through the reduction of caveolin 1 endothelial. Similarly, Jansen et al.¹⁷⁰ demonstrated that high glucose-induced L-EVs impair endothelial function as determined by a lower endothelial-dependent relaxation in EV-treated mice and by an increased adhesion of monocytes to L-EV-treated human coronary artery endothelial cells partly due to an up-regulation of

iCAM-1 and vCAM-1 in the treated cells. Similarly, L-EVs released from activated endothelial cells, such as those released during the transient diet-induced inflammation and endothelial dysfunction, exert a dual role. Luna et al.¹⁷¹ reported that endothelial L-EVs induced by inflammation alter the function of endothelial cell progenitors whereas Mahmoud et al. showed that they reduce oxidative stress and prevent lipid-induced endothelial damage¹⁷². On another note, it has been observed in the Framingham Offspring cohort the association of circulating level of endothelial L-EVs (referred as CD31, CD144⁺ EVs and known to reflect damaged endothelium) with the presence of cardiometabolic risk factors, particularly dyslipidaemia¹⁷³.

Other EVs subtypes (e.g. from adipose tissues, skeletal muscles), not detected so far in human plasma in response to a meal intake or a chronic dietary supplementation, participate to attenuate glucose intolerance and to improve insulin resistance¹⁷⁴⁻¹⁷⁷. Wu et al.¹⁷⁸ demonstrated that S-EVs derived from liver and enriched in miR-130a contribute to the recovery of insulin resistance induced by an HFD in mice. A study by Kumar et al.¹⁷⁹ revealed that an HFD affect intestinal epithelial S-EVs that are enriched in phosphatidylcholine resulting in an inhibition of the insulin response in mice. The authors report the same alteration in lipid composition in S-EVs from patients with T2DM. Together, these results reveal that S-EVs are potential contributors to the development of insulin resistance and that diet can be an efficient strategy to attenuate their impact.

In absence of more investigations on the biological function(s) of diet-induced EVs, available data support that unbalanced diet could drive through metabolic changes (glycaemia, lipaemia, oxidative stress and related inflammation) the release of EVs that convey pro-inflammatory, pro-thrombotic and pro-atherogenic messages, these latter representing in case of chronicity an increased risk for health. However, this risk could be attenuated by an improved diet supplying protective micronutrients and plant food bioactives.

Conclusion

This review highlights the importance to consider diet as an important regulator both in postprandial and fasting conditions of EV biology and to develop further investigations to extend knowledge in this promising but still underexplored research field.

EVs modulated by diet have been identified using the same methods (i.e. surface markers) than for the detection of fasting EVs in patients with a diagnosis or prognosis purpose. By consequence, diet-associated EVs share similar phenotype characteristics with disease-associated EVs. Apart the “fast” feature during the postprandial period, the kinetic of release of diet-associated EVs has never been fully described. Consequently, their persistence in the bloodstream till a fasting state cannot be excluded. If so, this phenomenon could skew the use of fasting EVs as a reliable health biomarker and this aspect must be addressed properly. One bias of the methods used to explore human plasma EVs is to exclude S-EVs that may be modulated by diet and could be use as reliable nutritional biomarker as the L-EVs. Further, EV content, that is considered as a fingerprint of their cells of origin, has not been reported for diet-associated EVs. One critical question in this regard is how the cargo (e.g. proteins, miRNAs and lipids) of diet-associated EVs allow to differ them from EV released in health disturbances. In addition, an accurate characterization of diet-associated EV content will contribute to a better understanding of their biological functions. In 2021, Mantilla-Escalante et al.¹⁸⁰ report that the long-term consumption of a Mediterranean diet enriched in extra-virgin olive oil or nuts from participants of the PREDIMED study improves the number and modulates the miRNA profiles of circulating exosomes. This modulation could help to explain how such diet lead to beneficial health effects. Based on the biological functions reported for diseases associated-EVs or *in vitro* induced EVs sharing similar phenotype features, EVs induced after consumption of an unbalanced diet are assumed to constitute a risk factor for health, that could potentially be slow down by supplying diet with plant food bioactives protective for health. Answering these pending questions will contribute to substantiate the interest of EVs and their content as innovative tools to explore the relationship between nutrition and health.

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Table Legend:

Table 1: Cell surface markers used for the identification of EV subpopulations by flow cytometry.

Table 2: Clinical trials investigating the impact of acute dietary intakes on EVs during the postprandial period.

Table 3: Human intervention studies reporting the effects of (sub)chronic dietary intake on circulating EVs. C: control diet; I: nutritional intervention.

Legend figures

Graphical abstract: Summary of key aims reviewed

Figure 1: Potential effects of dietary nutrients and metabolites driving the biogenesis and the release of EVs.