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Phenolic compounds and plant extracts as potential natural anti-obesity substances

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ABSTRACT

One strategy to prevent obesity could consist in the inhibition of the pancreatic lipase (PL). In an attempt to find natural antiobesity agents, phenolic compounds (PCs) and plant extracts were investigated on PL activity. Epigallocatechin-3-gallate > kaempferol and quercetin were detected as potent PL inhibitors, with IC_{50} of 0.8, 13.4 and 21.5 μM, respectively. Plant extracts from green tea and grape seed also shown potent inhibitory effect. Selected PCs were then assayed in an *in vitro* model of simulated intestinal fat digestion, based on the lipolysis of triolein. In such conditions closer to physiological reality, resveratrol, but also epigallocatechin-3-gallate and quercetin reduced the triolein digestion to ±50%. This could delay or decrease *in vivo* fatty acid absorption by enterocytes. This work therefore suggests that some PCs, at concentrations easily reached in the intestine following ingestion of tea beverages, fruits or vegetables, but also flavonoid-enriched supplements or functional food, are potential candidates for obesity prevention.

1. Introduction

Obesity consists in an excess of body fat resulting from an imbalance between energy intake and expenditure. It is a growing worldwide health problem considered as a risk factor for chronic diseases including e.g. cardiovascular diseases, type 2 diabetes, hypertension, stroke, mental health disorders and some forms of cancer. Insufficient physical activity, coupled with high-calorie, high-fat food consumption, is an environmental factor regarded as the main cause of obesity, beside genetic predisposition (Colagiuri, 2010; Robinson & Niswender, 2009). Although prevention with lifestyle modifications is obviously the most appropriate approach, therapeutic strategies comprise antiobesity agents and surgery. Only two drugs are currently approved for long-term obesity treatment, even though both of them have undesirable side effects. They either regulate food intake by acting on neural circuits (sibutramine™) or reduce nutrient absorption from gut (orlistat™). Consequently, there is a need for weight-loss effective and safe compounds (Chakrabarti, 2009; Robinson & Niswender, 2009). At present, the potential of natural products is largely unexplored and might be an alternative strategy for the development of antiobesity approaches. Among phytochemicals, "generally recognized as safe", with no side effects at plausible concentrations, the evidence that phenolic compounds (PCs) have beneficial effects in fighting or preventing obesity is increasingly reported, underlying their potential interest for a nutritional approach (Birari & Bhutani, 2007; Hsu & Yen, 2008).

The pancreatic lipase (PL) is the major enzyme responsible for the digestion of 50–70% of dietary triglycerides into monoacylglycerides and free fatty acids, both absorbable by enterocytes. The inhibition of PL could therefore result in a reduced fat absorption, and thereby energy uptake, which is one of the key targets thought to mediate obesity (Chakrabarti, 2009). Orlistat™ was shown to act by this mechanism (Hadvay, Lengsfeld, & Wolfer, 1988). Polyphenolic-rich extracts from teas, herbal and fruit sources, such as berry, apple, Melinjo or grape seeds, oolong, black or green tea from different varieties, various high fat- or high tannin-content plants, were reported as PL inhibitors during *in vitro* experiments. PCs such as proanthocyanidins or ellagitannins, gallate esters (gallocatechin gallate, epigallocatechin gallate (EGCG), catechin gallate) or stilbenoids (gnetin), were considered as the main active ingredients (Birari & Bhutani, 2007; He, Lv, & Yao, 2006; Horigome, Kumar, & Okamoto, 1988; Kato, Tokunaga, & Sakan, 2009; Kurihara, Asami, Shibata, Fukami, & Tanaka, 2003; Kusano et al., 2008; McDougall, Kulkarni, & Stewart, 2009; Nakai et al., 2005; Sugiyama et al., 2007). However, the inhibitory mechanism of these substances on PL remains unclear. It was generally attributed to the ability of tannins to bind, complex and precipitate proteins although some studies reported non-competitive or mixed inhibitions of PL (Gholamhoseinian, Shahouzehi, & Sharifi-far, 2010; Won, Kim, & Kim, 2007).

In this context, this study aimed at investigating the effect of individual PCs as well as plant extracts on PL activity, in an attempt to find non-toxic potential alternatives to pharmaceutical treatments or to provide nutraceutical prevention for obesity

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management. In a first set of experiments, a common enzymatic assay based on the hydrolysis kinetic of an oleate ester of 4-methylumbelliferone by the porcine PL was used to screen the effect of various PCs representative of the main chemical classes, *i.e.* ellagic, ferulic and gallic acids (phenolic acids), resveratrol (stilbene) and flavonoids from major groups, *i.e.* catechin, epicatechin and EGCG (flavanols), naringenin (flavanone), genistein (isoflavone), chrysin and luteolin (flavones), kaempferol, quercetin and quercetin 3-β-D-glucoside (Q3BCG) (flavonols), as well as punicalagin (tannin) and curcumin. Selected PCs were then assayed in conditions closer to the physiological reality, in an *in vitro* lipid digestion model based on the hydrolysis of a triglyceride (triolein) by porcine pancreatin, containing lipase and co-lipase, in the presence of a bile extract. In the two approaches, the initial lipolysis rate was followed in order to simulate the *in vivo* situation. Indeed, during pancreatic digestion of triglycerides, the hydrolysis products are continuously removed being absorbed by enterocytes. This process generates conditions producing a maximal lipolysis rate.

2. Materials and methods

2.1. Chemicals

Catechin, chrysin, curcumin, ellagic acid, epicatechin, EGCG, ferulic acid, gallic acid, genistein, kaempferol, luteolin, naringenin, quercetin, quercetin 3-β-D-glucoside, resveratrol, lipase from porcine pancreas (type II), 4-methylumbelliferyl oleate, 4-methylumbelliferone, pancreatin from porcine pancreas, Tris-maleate, bile extract porcine, triolein, were from Sigma–Aldrich (St. Louis, MO) and punicalagin from PhytoLab (Vestenbergsgreuth, DE). Folin–Ciocalteu's phenol reagent was obtained from VWR International (Leuven, BE).

2.2. Plant extracts

Extracts from green tea (*Camelia sinensis*), grape seeds (*Vitis vinifera*), oak duramen (*Quercus robur*) and pomegranate peel (*Punica granatum*) were provided by Stiernon SA/NV (Ath-Ghislenghien, BE). Accordingly to the manufacturer suggestion, solutions of 5 mg of extract/ml were prepared in water at room temperature in the dark during 2 h with agitation. They were then filtrated (Whatman 1) (Whatman, Brentford, UK) and the solutions were stored at −20 °C under a nitrogen atmosphere.

2.3. Estimation of total phenolics

Total phenolic concentration of plant extract solutions was estimated using the Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1999). 50 µl of appropriated diluted samples, 1250 µl of a sodium carbonate solution at 7.5% (w/v) and 250 µl of Folin–Ciocalteu reagent were mixed and allowed to react at room temperature for 30 min. The absorption at 755 nm was then measured using a Beckman DU 800 spectrophotometer (Fullerton, CA). A standard curve was made in parallel with a gallic acid standard. Total phenolic concentration was expressed as µM of gallic acid equivalents (GAE).

2.4. Quantification of phenolic compounds by UPLC–MS/MS

The plant extract solutions were dried in a Thermo Scientific SpeedVac concentrator (Thermo Fisher Scientific Inc, Waltham, MA) and solubilized in methanol/water (1:1, v:v). Compounds were separated in an Acquity BEH shield column 2.1 × 100 mm using an Acquity UPLC system fitted with a photodiode array detector and a triple quad mass spectrometer (Waters, Milford, MA). The ionization source was used in the ESI negative mode. MassLynx (Waters) was used as software. The eluting phase was a gradient of acetonitrile and ammonium acetate buffer, pH 2.6.

Compounds were identified by comparison with authentic standards of phenolic compounds.

2.5. PL activity assay

The PL activity was assessed by measuring the rate of release of 4-methyumbelliferone (4MU), using 4-methylumbelliferyl oleate (4MUO) as substrate. The assay was adapted from Kurihara et al. (2003). In brief, 25 µl of a blank, *i.e.* DMSO or water as PC or plant extract vehicle, respectively, or a sample solution and 50 µl of 0.5 mM 4MUO, each diluted from their stock solution in a buffer consisting of 13 mM Tris–HCl, 150 mM NaCl, and 1.3 mM $CaCl_2$ (pH 8.0), were mixed in 96-well microtiter plate. 25 µl of lipase from porcine pancreas (Type II), prepared at 0.5 mg/ml in the same buffer, was then added to start the enzyme reaction. After incubation at room temperature for 0, 60, 90 and 150 s, 100 µl of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. Fluorescence, corresponding to released 4MU, was then measured in a Fluoroskan Ascent fluorimeter (Thermo Electron Corp., Boston, MA) with excitation and emission wavelengths of 355 and 460 nm, respectively. The quantity of 4MU was determined from a standard curve. The PL activity was expressed as pmoles of 4MU produced per min, calculated from the initial rate.

2.6. In vitro lipid digestion

The PL activity was assessed with a second, more physiologic, method by measuring the rate of release of oleic acid from triolein. The assay was adapted from Sek, Porter, Kaukonen, and Charman (2002). A fresh pancreatin solution was prepared by mixing 1 g of pancreatin from porcine pancreas to 5 ml of digestion buffer, consisting in 150 mM NaCl, 5 mM $CaCl_2$, 50 mM Tris-maleate, pH 7.0. This mixture was stirred for 15 min followed by centrifugation at 1600*g* for 15 min. The supernatant was collected and stored at 4 °C.

Fifteen milligram of porcine bile extract were dissolved in 5 ml of digestion buffer. 6.4 mg of triolein were then mixed with 700 µl of the bile solution during 15 min at 37 °C before adding 100 µl of pancreatin solution. Digestion was stopped after 0, 150, 300 and 450 s, by rapid freezing before lipid analysis. The PL activity was calculated from the initial rate as the amount of free oleic acid liberated per min.

2.7. Lipid analysis

Total lipids were extracted with chloroform/methanol/water (2:2:1.8, v:v:v) (Bioslove, Valkenswaard, NL) according to the method of Bligh and Dyer (1959). The extracts were then separated by Solid Phase Extraction (Bond Elut–NH_2, 200 mg, 3 ml; Varian, Palo Alto, CA) into three lipid fractions, *i.e.* neutral lipids (NLs), free fatty acids (FFAs) and phospholipids (PLs). A distinct internal standard was used for each collected fraction, in order to quantify the fatty acids present in the different fractions. More precisely, a known amount of triheptadecanoin (C17:0; Larodan, Malmö, SE), tridecanoic acid (C13:0; Sigma–Aldrich) and 1,2-dipentadecanoyl-sn-glycero-3-phosphatidylcholine (C15:0; Larodan) was added to each sample before lipid extraction for quantification of NL, FFA and PL, respectively. After conditioning the columns with 3 ml of hexane (Biosolve), the total lipid extract dissolved in 200 µl of chloroform was loaded. After the chloroform was pulled through, the NLs were eluted with 1.8 ml of chloroform/2-propanol (2:1, v:v) (Biosolve). The column was then loaded with 2.4 ml of diethyl ether/acetic acid (98:2, v:v) (Bioslove) followed by 1.8 ml

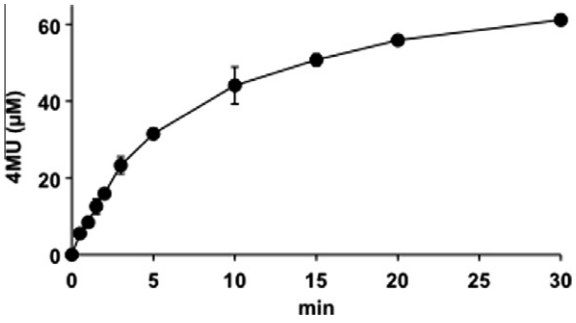

Fig. 1. Kinetic of 4-methyumbelliferone (4MU) liberation by the porcine pancreatic lipase, from 4MUO as substrate. Data represent means ± SEM from three independent experiments each having three replicates per condition.

of methanol (Biosolve) to elute the FFAs and PL, sequentially. Each fraction was then dried under nitrogen and methylated at 70 °C through the addition of 1 ml of 0.1 M KOH in methanol and a 1 h incubation followed by the addition of 0.4 ml of 1.2 M HCl in methanol and incubation during 15 min. The fatty acid methyl esters were then extracted by 2 ml of hexane.

The fatty acid methyl esters were then separated by GC. The GC Trace (Thermo Finnigan, Milan, IT) gas chromatograph was equipped with a RT2560 capillary column (100 m × 0.25 mm internal diameter, 0.2 µm film thickness; Restek, Bellefonte, PA), a GC PAL autosampler (CTC analystics, Zwingen, CH) and a flame ionization detector (FID). The carrier gas used was H_2 at a constant pressure of 200 kPa. The FID was kept at a constant temperature of 255 °C. The temperature program was as follows: an initial temperature of 80 °C, which progressively increased at 25 °C.min^{-1} up to 175 °C; a holding temperature of 175 °C during 25 min followed by an increase at 10 °C min^{-1} up to 205 °C; a holding temperature of 205 °C during 4 min followed by a new increase at 10 °C min^{-1} up to 225 °C and a holding temperature of 225 °C during 20 min. A decrease at 20 °C min^{-1} was then imposed down to the initial temperature of 80 °C.

2.8. Data analysis

Results were expressed as means ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). The computer program was StatPlus 5.7.5.0 (AnalystSoft Inc., Vancouver, CAN). Results with a two-sided *P*-value < 0.05 were considered statistically significant.

3. Results

3.1. Inhibition of the PL activity by PCs

The kinetic of 4MUO hydrolysis by the porcine PL was firstly established. As shown Fig. 1, the 4MU production rate was constant during the first couple of minutes of the reaction, *i.e.* until 3 min. This time interval, corresponding to the initial hydrolysis rate, was chosen for the later PL activity measurements.

Various dietary PCs, representative of the main chemical classes, were selected and tested for their potency to inhibit the PL activity: ellagic, ferulic and gallic acids (phenolic acids), resveratrol (stilbene) and flavonoids from major groups, *i.e.* catechin, epicatechin and EGCG (flavanols), naringenin (flavanone), genistein (isoflavone), chrysin and luteolin (flavones), kaempferol, quercetin and quercetin 3-β-D-glucoside (Q3BCG) (flavonols), as well as punicalagin (tannin) and curcumin. The PCs were assayed at a final concentration of 100 µM and the lipase activity was expressed as a percentage of the control corresponding to the enzyme incubated in the presence of the PC vehicle (DMSO). DMSO was detected as having no effect on the PL activity (data not shown). As presented in Fig. 2, some PCs significantly reduced the PL activity: EGCG inhibited the PL activity almost completely. Curcumin and the 3 flavonols, *i.e.* kaempferol, quercetin and Q3BDG, were also potent inhibitors leading the lipase activity at 17.8 and *e.g.* 23%, respectively, of the control value. Luteolin, resveratrol, punicalagin and ferulic acid, had an average inhibition level of 50%. Ellagic acid, as well as genistein, exhibited only a 30% inhibitory effect. Gallic acid, naringenin, catechin, epicatechin and chrysin, had no significant effect.

The inhibitory PCs were then assayed at different concentrations and detected as reducing the PL activity in a dose-dependent manner (Fig. 3). Their IC_{50} (concentration resulting in 50% inhibition) calculated from the inhibition curves are presented Table 1. Orlistat™, a potent and specific lipase inhibitor (Hadvay et al., 1988), was also tested as a positive control and displayed an IC_{50} of 32 µM (18.5 mg/l). EGCG and kaempferol, with an IC_{50} of 0.8 and 13.4 µM, respectively, were more efficient than orlistat™ to inhibit the PL activity. Quercetin and curcumin had an inhibitory effect of same magnitude as the drug.

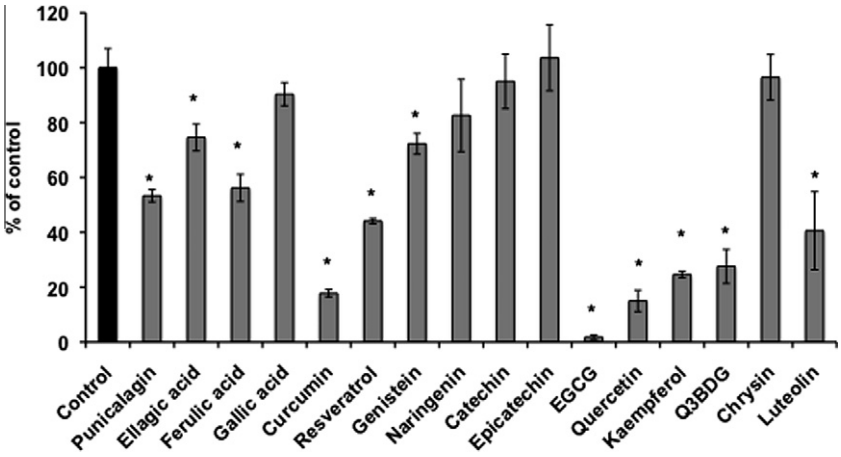

Fig. 2. Effect of phenolic compounds (PCs) on the porcine pancreatic lipase activity. The lipase activity was determined using 4MUO as substrate, after incubation with different PCs at 100 µM or their vehicle (DMSO) as control. Results, calculated from initial rates as pmoles of 4MU produced × min^{-1}, were expressed as percentage of the control. Data represent means ± SEM from three independent experiments each having three replicates per condition. * Indicates $P < 0.05$ as compared to the control condition.

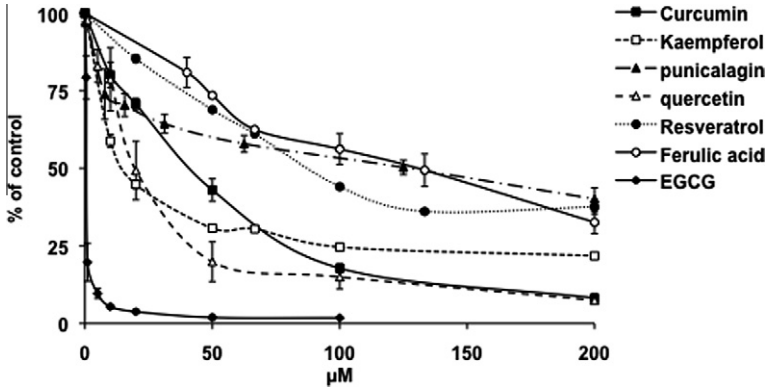

Fig. 3. Dose–response of phenolic compounds (PCs) on the porcine pancreatic lipase activity. The lipase activity was determined using 4MUO as substrate, after incubation with different PC concentrations from 1 to 200 µM. Results, calculated from initial rates as pmoles of 4MU produced × min^{-1}, were expressed as percentage of the control. Data represent means ± SEM from three independent experiments each having three replicates per condition.

Table 1
Inhibitory effect of PCs and orlistat™ on the pancreatic lipase activity.

	IC_{50} (µM)[a]
EGCG	0.8 ± 0.1
Kaempferol	13.4 ± 4.1
Quercetin	21.5 ± 9.4
Curcumin	43.9 ± 12.6
Resveratrol	90.7 ± 8.7
Ferulic acid	123.9 ± 13.4
Punicalagin	125 ± 2.3
Orlistat™	32.0 ± 8.5

[a] IC_{50} were determined from inhibition curves realized by measurement of the porcine pancreatic lipase activity, using 4MUO as substrate, in the presence of different PC or orlistat™ concentrations from 1 to 200 µM. Data represent means ± SEM from three independent experiments each having three replicates per condition.

Plant extracts were also assayed to evaluate their potential PL inhibitory effects. Green tea, grape seeds and oak extracts were characterized by UPLC–MS–MS for their phenolic content (Table 2). Green tea and grape seed mainly contained condensed tannin compounds, i.e. catechin, epicatechin and EGCG, whereas ellagic and gallic acids were the principal phenolics of oak extract. Pomegranate extract was shown to contain high amounts of punicalagin and ellagic acid, as well as gallic acid (Hollebeeck, Leclercq, Winand, Larondelle, & Schneider, submitted for publication). To match with the physiological reality, the extracts were firstly solubilized in water. Indeed, the extracts are intended to be use as food supplements and, as such, they will end up in the gastrointestinal liquids, an aqueous environment restricting the compound bioaccessibility. Plant extract solutions were tested at various concentrations, on the basis of their estimated phenolic content expressed in gallic acid equivalents (GAE), which should allow comparison between extracts. The extracts exhibited a dose-dependent inhibitory effect on the PL activity and their IC_{50} have been calculated (Table 3). Green tea and grape seed extracts had potent inhibitory effect, showing IC_{50} values smaller than that of orlistat™. Oak and pomegranate extracts were detected as less efficient with IC_{50} > orlistat™.

3.2. Inhibition of the in vitro lipid digestion

In order to mimic the digestion in more realistic conditions, from a physiological point of view, we investigated the effects of PCs on lipid pancreatic digestion in an *in vitro* model simulating some of the conditions in the duodenum. Triolein, as a model triglyceride, was mixed with bile extract that contains bile salts and phospholipids, and pancreatin comprising pancreatic lipase and co-lipase. In such conditions, the effect of some PCs, used at 100 µM, was assayed on the liberation of oleic acid. As shown Fig. 4, resveratrol significantly reduced the oleic acid release to 46% of the control. EGCG and quercetin also had a significant decreasing effect on the triolein digestion whereas curcumin and

Table 2
Phenolic compounds (mg/g of extracts) as determined by HPLC-MS/MS.

Phenolic compounds	MS/MS transitions	Green tea[a]	Grape seed[a]	Oak[a]
Catechin	289.18 > 122.58 289.18 > 245.04	2.8 ± 0.1	58.2 ± 0.9	n.d.
Ellagic acid	301.12 > 144.96 301.12 > 284.01	0.3 ± 0.0	0.9 ± 0.0	31.6 ± 1.5
Epicatechin	289.06 > 109.05 289.06 > 244.99	16.9 ± 0.4	35.7 ± 1.2	n.d.
EGCG	457.06 > 124.99 457.06 > 168.94	103.4 ± 3.2	n.d.	n.d.
Gallic acid	169.11 > 79.08 169.11 > 125.00	2.3 ± 0.1	5.3 ± 0.1	26.3 ± 0.9
Resveratrol	277.15 > 143.02 277.15 > 185.08	n.d.	n.d.	n.d.
Quercetin	301.13 > 151.02 301.13 > 178.96	0.4 ± 0.0	n.d.	n.d.

n.d. = not detected.
[a] Values are means ± SD.

Table 3
Inhibitory effect of plant extracts on the pancreatic lipase activity.

	IC_{50} (μM GAE)[a]
Green tea	2.8 ± 0.3
Grape seed	3.8 ± 0.3
Oak	144.0 ± 13.1
Pomegranate	>300

[a] IC_{50} were determined from inhibition curves realized by measurement of the porcine pancreatic lipase activity, using 4MUO as substrate, in presence of plant extract concentrations from 1 to 500 μM of gallic acid equivalents (GAE). Data represent means ± SEM from three independent experiments each having three replicates per condition.

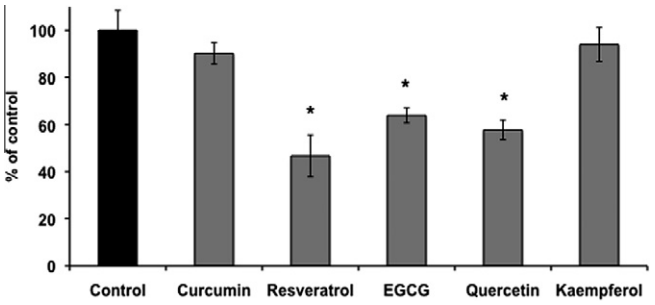

Fig. 4. Effect of phenolic compounds (PCs) on the *in vitro* lipid digestion. The lipase activity was determined using triolein as substrate, after incubation with different PCs at 100 μM or their vehicle (DMSO) as control. Results, calculated from initial rates as the quantity of oleic acid recovered in the free fatty acid fraction, were expressed as percentage of the control. Data represent means ± SEM from three independent experiments. ∗ indicates $P < 0.05$ as compared to the control condition.

kaempferol had no effect. Orlistat™, at 80.7 μM (40 mg/l), a realistic intestinal drug concentration, reduced the FA release to 37% of the control value.

4. Discussion

This study aimed at investigating the inhibitory effect of various PCs, representative of the main chemical classes and widely found in food or dietary supplements, as well as some plant extracts, on the PL activity in order to find potential natural anti-obesity agents.

Firstly, using a fast procedure based on the 4MUO hydrolysis by porcine PL, we detected that EGCG, curcumin and three flavonols, *i.e.* kaempferol, quercetin and Q3BDG, had a strong inhibitory effect. Other PCs, such as punicalagin, ellagic and ferulic acids, resveratrol, genistein and luteolin, also had a significant, but weaker inhibitory effect, on the PL activity whereas gallic acid, naringenin, catechin, epicatechin and chrysin were unactive, which suggests the absence of direct structure–activity relationship. The inhibitory effect of EGCG on PL activity was already observed (Kusano et al., 2008; Nakai et al., 2005). Nakai et al. (2005) reported an IC_{50} of 0.35 μM for EGCG, which is of same order of magnitude than our results, *i.e.* 0.8 μM. For comparison, in our assay, orlistat™, the most common anti-obesity drug, had an IC_{50} of 32 μM, being therefore 40-fold less effective than EGCG. EGCG is a catechin abundant in green tea and concentrations up to 100 μM can be reached in the intestine following the ingestion of green tea infusion (Scalbert & Williamson, 2000). Its effect on PL was attributed to its galloyl moiety (Nakai et al., 2005). EGCG, used as a tannin model, was already reported to form aggregate with proteins, e.g. human salivary proline-rich proteins (Pascal, Paté, Cheynier, & Delsuc, 2009). A tannin effect could therefore be implicated in the PL inhibitory mechanism of EGCG. Such mechanism was already proposed to explain its inhibitory effect on the activity of another digestive enzyme, *i.e.* the phospholipase A_2 (Wang, Noh, & Koo, 2006). Kaempferol, quercetin, as well as curcumin, have also shown potent inhibition of PL activity, with their $ICs_{50} \leqslant IC_{50}$ of orlistat™. Kaempferol and quercetin are the main representative flavonols present in our diet, commonly found in apples, onions, curly kale, broccoli and green tea, and curcumin is the major constituent of the curcuma spice (Aggarwal & Harikumar, 2009; Boots, Haenen, & Bast, 2008). Due to their potential therapeutic properties, these PCs are also found in countless food supplements. Inhibitory concentrations should therefore be easily reached in the intestine. Although these PCs are described as binding proteins (Barik, Priyadarsini, & Mohan, 2003; Cao, Liu, Shi, Xiao, & Xu, 2008), there are no reports on their ability to form aggregate, which suggests that their mode of action on PL should be more specific than that of EGCG. In our study, resveratrol was also detected as a PL inhibitor, although less potent than orlistat™, with an IC_{50} of 90.7 μM. In contradiction with our results, Kato et al. (2009) described this stilbene as having no inhibitory activity against PL, with an $IC_{50} > 200$ μM. This discrepancy could be due to differences in the PL activity assay such as the duration of incubation (30 min (Kato et al., 2009) vs 2.5 min) and/or the resveratrol stability.

Most studies on PL inhibition have been carried out on polyphenol-rich extracts, mainly from teas, or on fractions isolated from these extracts. Their high PL inhibitory activity was usually attributed to the condensed tannin content, *i.e.* the proanthocyanidins, mainly those having functional galloyl moieties as found in EGCG or GCG subunits (Birari & Bhutani, 2007; He et al., 2006; Horigome et al., 1988; Nakai et al., 2005; Sugiyama et al., 2007), or to ellagitannins (berry extract) (McDougall et al., 2009). Tannins, including proanthocyanidins, can bind to various proteins and aggregate them (Hagerman & Butler, 1981). The tannin–protein bound is generally considered to be nonspecific (Haslam, 1996). In this context, He et al. (2006) reported that tea polyphenols might act as antinutritional factor on digestive enzymes. Our results with the plant extracts agree with an implication of condensed tannins content in the PL inhibition. Indeed, the potent inhibitory effect of green tea can be largely attributed to EGCG, representing *ca.* 60% of total phenolic content of the extract. In the grape seed extract, catechin and epicatechin, two flavan-3-ols that exhibited no effect on the lipase activity, were detected as the main phenolics. However they represent only 17% of the total phenolic content. This implies the presence of other compounds, undetectable by our method, such as proanthocyanidins, *i.e.* oligomers and polymers of catechin and epicatechin, which can account for 80% of grape seed extract (Gabetta et al., 2000) and have been implicated in the lipase inhibition (Moreno et al., 2003; Nakai et al., 2005; Sugiyama et al., 2007). Besides, oak and pomegranate are also tannin-rich extracts, but they did not display potent PL inhibition. These extracts mainly contain hydrolysable tannin compounds, i.e. ellagic and gallic acids and punicalagin, detected in this study as weak or nil inhibitor of the PL activity. It should also be mentioned that the PL used during our assays, but also in most studies of the literature, was not a pure enzyme but a crude extract, described by the manufacturer as containing some amylase and protease activity. This complicates the result interpretation since PC can also inhibit the other digestive enzymes and, moreover, since the assay substrate, the 4MUO, is not specific of lipases. Therefore, more relevant investigations have been next realized.

Secondly, some selected PCs were assayed in conditions closer to the physiological reality in an *in vitro* model of simulated intestinal lipid digestion, using triolein (glycerol trioleate) as substrate, upon addition of pancreatin and bile extract. At 100 μM, resveratrol was the most effective, decreasing the lipid digestion to 50% of the control. EGCG and quercetin, detected as potent PL inhibitors in the enzymatic assay, showed weaker, but still significant,

inhibitory effect on the lipid digestion. This could result from interactions between these flavonoids and other components present during the *in vitro* digestion, such as other digestive enzymes of the pancreatin, *e.g.* the phospholipase A_2 (Wang et al., 2006), or bile acids, preventing in this case the lipid emulsification (Koo & Noh, 2007). Beside, PCs could also be degraded during the pancreatic digestion. Indeed, some studies reported a loss of EGCG, up to 90% from green tea water extracts, and a 26% decrease for quercetin during an *in vitro* digestion (Boyer, Brown, & Liu, 2005; Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). Resveratrol degradation was also reported under pancreatic conditions in a study on the grape polyphenol bioaccessibility (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). This could argue in favor of a specific action of resveratrol on lipid digestion.

Resveratrol is reported as a health and disease benefit agent, found in grapes and red wine, peanuts, cranberries, strawberry, and also in dietary supplements (Tosun & Inkaya, 2010). Particularly, recent animal studies point the potential possibility of using resveratrol in preventing and/or treating obesity and diabetes: resveratrol was shown to act on different lipid parameters by reducing *i.e.* the plasma triglyceride, FFA and cholesterol levels, the visceral fat index or the body weight gain, as well as the adiposity, by a mechanism related to gene induction and enzyme activation (Szkudelska & Szkudelski, 2010). In the present study, we show that resveratrol could also act by another mechanism, *i.e.* by the inhibition of the triglyceride digestion that could result in reduced fatty acid absorption by enterocytes.

Although our results are preliminary and need further investigations, such as the influence of diet composition, using e.g. an *in vitro* digestion model, but especially *in vivo* assays and human intervention studies, this work provides new insights on the effect of PCs, mainly resveratrol but also EGCG and quercetin, as impairing the triglyceride digestion. This indicates that these PCs could be proposed as potential candidates in studies for developing functional foods or supplements that could contribute to obesity prevention. It also underlines the importance of using *in vitro* assays mimicking at best the physiological reality. In such context, *in vitro* digestion models simulating the pre-colonic digestion occurring during the nutrient transit from the mouth to the small intestine are particularly well appropriate to assess food safety and health benefits/risks linked to flavonoid-enriched supplements or functional food ingestion.

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