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Acute effects of an oral supplement of (−)-epicatechin on postprandial fat and carbohydrate metabolism in normal and overweight subjects

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Postprandial hyperglycemia, in particular when accompanied by excessive hypertriglyceridemia, is associated with increased cardiovascular risk, mainly in overweight or obese subjects, as it favors oxidative stress, systemic inflammation and endothelial dysfunction. Thus, treatments that favorably modulate metabolism by reducing steep increases in postprandial serum glucose and triglycerides, are of considerable interest. Evidence suggests that (−)-epicatechin (EPI) is responsible for reductions in cardiometabolic risk associated with chocolate consumption; these effects may be associated with favorable effects of EPI on postprandial metabolism. The aims of this study were to assess the effects of EPI on postprandial metabolism in normal-weight and overweight/obese subjects. Twenty adult volunteers (normal and overweight) underwent oral metabolic tolerance tests in the absence and presence of oral EPI (1 mg kg^{-1}). Metabolic responses were examined using indirect calorimetry and determining blood glucose and triglycerides at 0, 2 and 4 hours after metabolic load ingestion. Results show that EPI increased postprandial lipid catabolism, as evidenced by a significant decrease in the respiratory quotient, which implies an increase in fat oxidation. The effect was associated with significantly lower postprandial plasma glucose and triglycerides concentrations. The effects were more prominent in overweight subjects. In conclusion, EPI modulates postprandial metabolism by enhancing lipid oxidation accompanied by reductions in glycemia and triglyceridemia.

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1. Introduction

Postprandial metabolism, also known as *the fed state*, takes place after food ingestion and comprises a transient rise in glycemia and triglyceridemia due to the absorption of dietary glucose and fatty acids.[1] Normally, after a meal, glucose is rapidly metabolized, as it is the main energy substrate for the body; glucose is also stored, first as glycogen and, the excess, as fat. Fatty acids are almost entirely taken up into the liver, where they are re-synthesized into triglycerides and packaged into very low-density lipoproteins (VLDL). During the postabsorptive state, such metabolic processes become reversed; the previously stored glycogen and lipids are mobilized and oxidized in order to supply energy.

Postprandial hyperglycemia, especially when accompanied by excessive hyperlipemia (*i.e.*, serum triglycerides), has been reported to significantly increase the risk of developing cardiovascular (CV) diseases, even when, under fasting conditions, subjects show normal blood glucose and triglycerides.[2] Moreover, these phenomena are exacerbated in overweight subjects.

Hence it has been proposed that novel approaches aimed to reducing cardiometabolic risks may need to target improving postprandial metabolism, specifically by limiting steep increases in blood glucose and triglycerides.[3] Interestingly, metformin[4] and other agents, such as glucagon-like peptide analogs,[5] gliptins,[6] and acarbose,[7] have shown favorable effects in postprandial metabolism; however, the occurrence of significant side-effects limits their consideration as safe and effective for these purposes.

Nutraceutical agents, such as green tea extracts, have been considered for these effects. However, the large number of chemical constituents in green tea makes it difficult to identify the active components and to standardize them for their possible widespread use. Recent reports have evidenced an

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association between modest cocoa consumption and improved cardiometabolic health, demonstrating significant benefits on blood pressure, platelet reactivity, HDL-cholesterol, LDL-cholesterol, and insulin sensitivity, amongst others.[8] EPI is the most abundant flavanol in cocoa and the evidence indicates that it is the primary agent responsible for cocoa-induced improvements in the cardiometabolic profile.[8] Additionally, no toxicity has been reported for EPI and, in fact, it has been used as an attenuator for the effects of other toxic agents.[9] Interesting results have been found in studies involving the effects of dark chocolate,[10] flavonoid-rich foods or extracts,[11] and other purified flavanols[12] on postprandial metabolism. However, the possible use of EPI as a modulator of postprandial metabolism has not been explored.

With these issues in mind, we evaluated the effects of EPI on postprandial metabolism in normal-weight and overweight/obese subjects.

2. Methods

2.1 Study design

We conducted a pilot cross-over, open-labeled study in twenty volunteers. Inclusion criteria were: age (20–45 years) and body-mass index (BMI) >18.5 and <30 kg m^{-2}. Exclusion criteria included the presence of chronic disease that could disturb "normal" postprandial metabolism (*e.g.*, cancer, liver disease, *etc.*) and/or the use of dietary/pharmacologic agents that modulate postprandial metabolism (*e.g.*, acarbose and fiber supplements). Participants were eliminated from the study if they did not comply with all measurements and/or if they withdrew informed consent. Institutional Review Board (IRB) granted approval for the present protocol. All procedures followed ethical standards as stated in the Helsinki Declaration.

2.2 Anthropometrics

Measurements performed prior to testing included body weight, height, skinfolds – all previous according to Lohman's standardized procedures – and BMI calculation. Subjects were then classified, using the World Health Organization (WHO) criteria, as normal-weight (18–24.99 kg m^{-2}) or overweight (≥25 kg m^{-2}). As body composition, rather than total body mass, can affect systemic metabolism, the results were also segregated for a sub-analysis using fat mass as a group-classification criteria. For this purpose, adiposity was estimated by measuring skinfolds – suprailiac, bicipital, tricipital, and subescapular – and then calculating the body's density and, by using Siri's regression equations, reported as a fat mass percentage. Obesity (*i.e.*, high-adiposity) was diagnosed with the currently accepted cut-off points:[13] for men ≥25% and ≥32% for women.

2.3 Oral metabolic tolerance test (OMTT)

An initial blood sample (0 h) was collected by venipuncture into heparinized tubes and plasma separated; this was used to determine glucose and triglycerides, using commercially available kits (Randox® S. A., Mexico). Afterwards, indirect calorimetry was performed, *in vivo*, using an open-circuit system (Korr® CardioCoach CO_2) from which the respiratory quotient (RQ) was computed in order to determine the primary source of energy metabolism. Briefly, subjects were reclined in a comfortable position; they were then asked to place a padded nose clip on the nose. A MetaBreather® mouthpiece was provided to breathe into, and the subject was asked to make a good seal with his/her lips around the mouthpiece. Precautions in order to improve accuracy of indirect calorimetry were taken, including: resting for at least 30 minutes before study, 10 hours of fasting, no alcohol consumption nor intense exercising 48 hours prior to testing, emptying the bladder 30 minutes before the test, quiet and thermo-neutral environment, and ensuring of no leaks during measurements. Participants then ingested a commercial nutritional supplement (Ensure® Regular, 237 mL containing 39 g of carbohydrates, 9 g of protein, and 6 g of lipids, yielding 246 kcal with a 63-15-22 percentage macronutrient distribution) within a period no longer than 5 minutes. Two hours later, a second blood sample was obtained and calorimetry was repeated. The third and last blood sample and calorimetric evaluation were obtained at 4 hours. One week later, subjects repeated the OMMT with prior administration of EPI (Sigma Chemicals®, USA) at a dose of 1 mg kg^{-1}, 30 min prior to the OMTT study using gelatin capsules as carriers. The dose was selected on the basis of reports that demonstrate significant effects on cardiometabolic endpoints.[14]

Table 1 Basal characteristics[a]

Group	Gender (*n*, %)	Age (years)	Weight (kg)	BMI (kg m^{-2})	Glycemia (mmol L^{-1})	TG (mmol L^{-1})	RQ (VCO_2/VO_2)	kcal from fat (%)
Total	F:9 (45) M:11 (55)	27.8 ± 1.4	69.4 ± 3.2	24.0 ± 0.7	4.9 ± 0.1	1.2 ± 0.1	0.87 ± 0.02	45.4 ± 5.1
Normal BMI (<25 kg m^{-2})	F:7 (58.3) M:5 (41.6)	28.4 ± 2.2	63.8 ± 3.9	22.0 ± 0.5	4.8 ± 0.1	1.1 ± 0.1	0.86 ± 0.01	46.1 ± 4.9
Overweight (≥25 kg m^{-2})	F:2 (25) M:6 (75)	26.8 ± 1.1	77.8 ± 4.5*	27.1 ± 0.7*	4.9 ± 0.1	1.4 ± 0.1**	0.90 ± 0.05*	44.4 ± 10.9*

[a] TG: triglycerides; RQ: respiratory quotient *$p < 0.05$, **$p < 0.01$ (Student's independent *t*-test, normal *vs.* overweight groups).

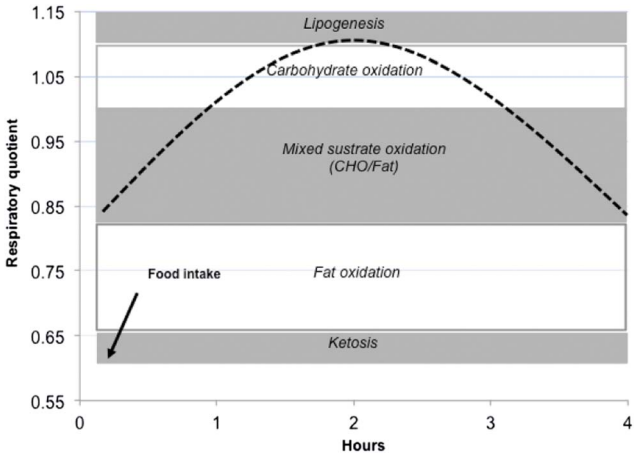

Fig. 1 Respiratory coefficient chart and theoretical curve switch in metabolism meal ingestion. The respiratory coefficients usually vary from 1.0 (representing the value expected for pure carbohydrate oxidation) to ~0.7 (the value expected for pure fat oxidation).

2.4 Statistical analysis

The results are presented as mean ± standard deviation, unless otherwise stated. Student's paired *t*-tests were performed for mean comparisons, whereas non-parametrical tests (*i.e.*, change in RQ, fat oxidation, glycemia, *etc.*) were used with non-continuous variables. A mixed model analysis was performed to explore differences in responses over time *vs.* baseline values. A p value <0.05 was considered as statistically significant. The statistical package herein used was GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

Twenty volunteers divided according to their BMI (12 normal and 8 overweight subjects) were included in this study. Table 1 shows their anthropometric and basal metabolic characteristics, respectively. In agreement with metabolic disturbances that are known to be present amongst overweight/obese subjects, basal RQ ($p < 0.05$) and triglyceridemia were significantly ($p < 0.01$) higher, while fat oxidation ($p < 0.05$) was significantly lower in comparison with normal-weight subjects.

3.1 Calorimetry

Fig. 1 illustrates a theoretical RQ curve after the intake of any meal. Further, Fig. 2 shows the results from indirect calorimetry for normal (A) and overweight subjects (B). Fig. 2A shows that in the absence of EPI, amongst normal-weight subjects; basal fasting RQ values (0.86 ± 0.01) reflect a mixed substrate oxidation of carbohydrates and fats. After the meal ingestion, the RQ rose transiently to 0.89 ± 0.01 as dietary glucose becomes the short-term energy substrate. By 4 h, RQ dropped back to 0.83 ± 0.01, in accordance with the metabolic switching towards fat oxidation. In the overweight subjects, as

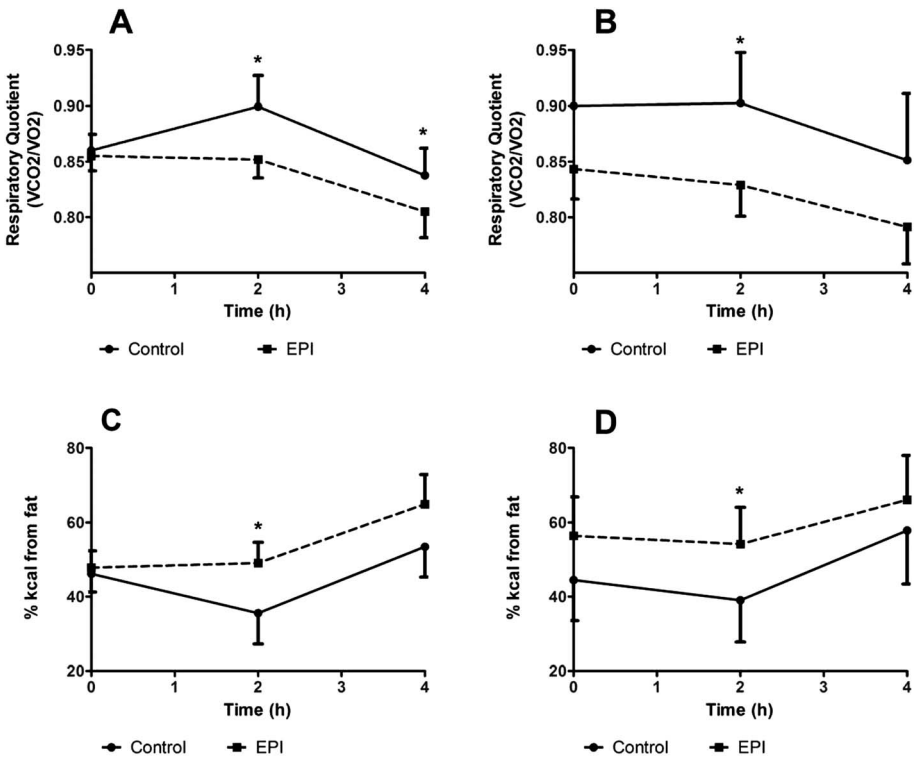

Fig. 2 Respiratory quotient values and estimated percent kilocalories obtained from fat oxidation in (A and C) normal ($n = 12$) and (B and D) overweight ($n = 8$) subjects, recorded at 0, 2 and 4 hours during the OMTT. Data are expressed as mean ± SD, p values by paired t-tests, where *p < 0.05. As seen, the postprandial increase in RQ was significantly attenuated and lipid catabolism was enhanced by EPI in both groups.

shown in Fig. 2B, metabolic changes were also noted although the baseline RQ was significantly higher (0.90 ± 0.05 *vs.* 0.86 ± 0.01, $p < 0.05$) than that of the normal-weight subjects thus, evidencing impaired fat oxidation. The 2 h postprandial RQ value was unaltered (0.90 ± 0.04) and, by 4 h, dropped to 0.85 ± 0.06.

With EPI supplementation, fat oxidation at fasting (*i.e.*, 0 h) was unaltered in normal-weight subjects as the RQ was 0.85 ± 0.01. However, by 2 h, the RQ remained within similar values, almost unchanged, in comparison with the fasting state but was significantly lower in comparison with the result noted in the absence of EPI (0.85 ± 0.01 *vs.* 0.89 ± 0.01, $p < 0.05$), suggesting that EPI sustained fat oxidation even after the nutritional load. By 4 h, the RQ decreased even more *versus* both 2 prior hours and was significantly lower (0.80 ± 0.02 *vs.* 0.85 ± 0.06, $p < 0.05$) than the values recorded without EPI. In overweight subjects, a greater effect of EPI was found as the RQ was significantly lowered at 0 h (0.84 ± 0.02 *vs.* 0.90 ± 0.05, $p < 0.05$), 2 h (0.82 ± 0.02 *vs.* 0.90 ± 0.04, $p < 0.05$), and 4 h (0.79 ± 0.03 *vs.* 0.85 ± 0.06, $p < 0.05$) after meal intake.

Accordingly, as illustrated in Fig. 2, lipid oxidation, represented as percentage of kilocalories obtained from fat, was significantly higher at 2 h, by EPI supplementation, in normal (C) and overweight (D) subjects.

We also compared the percent change in kcal obtained from fat 2 h after meal ingestion *versus* fasting conditions (time 0) in the absence or presence of EPI. In normal subjects, there was a trend to increase net fat oxidation (Fig. 3A), while a statistically significant increase in lipid oxidation was observed in overweight volunteers (Fig. 3B), showing that EPI does not only attenuate the physiologic postprandial drop in fat metabolism but, in fact, increases it.

3.2 Blood chemistry

The results are summarized in Table 2. Values recorded in the absence of EPI, corresponded to those of normal fed-state metabolism as blood glucose and triglycerides increased after meal ingestion and decreased afterwards.

With EPI administration in normal subjects, significant decreases in 4 h glucose (4.7 *vs.* 4.3 mmol L^{-1}, $p < 0.05$) and 0 h triglycerides (1.1 *vs.* 0.8 mmol L^{-1}, $p < 0.05$) were observed. In the overweight group, with EPI, significant decreases in fasting glycemia (4.9 *vs.* 4.2 mmol L^{-1}, $p < 0.05$) and postprandial triglyceridemia (2.0 *vs.* 1.6 mmol L^{-1}, $p < 0.01$) were observed.

In the mixed model analysis in the presence of EPI, a significant decrease in glucose and triglycerides levels occurred 2 h after meal ingestion.

Table 2 Postprandial metabolism blood values[a]

	Total	
	Control (no EPI)	EPI
Time (h)	**Glycemia (mmol L^{-1})**	
0	4.9 ± 0.1	4.5 ± 0.1**
2	5.7 ± 0.2	5.2 ± 0.1*
4	4.9 ± 0.1	4.3 ± 0.1**
Time (h)	**Triglyceridemia (mmol L^{-1})**	
0	1.2 ± 0.1	1.0 ± 0.1
2	1.6 ± 0.1	1.3 ± 0.1**
4	1.3 ± 0.1	1.1 ± 0.1

	Normal BMI (<25 kg m^{-2})		Overweight (≥25 kg m^{-2})	
	Control (no EPI)	EPI	Control (no EPI)	EPI
Time (h)	**Glycemia (mmol L^{-1})**			
0	4.8 ± 0.1	4.6 ± 0.2	4.9 ± 0.2	4.2 ± 0.1*
2	5.5 ± 0.1	5.2 ± 0.2	5.9 ± 0.3	5.2 ± 0.2
4	4.7 ± 0.2	4.3 ± 0.1*	4.9 ± 0.2	4.3 ± 0.2
Time (h)	**Triglyceridemia (mmol L^{-1})**			
0	1.0 ± 0.1	0.8 ± 0.05*	1.4 ± 0.1	1.3 ± 0.3
2	1.3 ± 0.1	1.1 ± 0.09	2.0 ± 0.2	1.6 ± 0.2**
4	1.0 ± 0.1	0.9 ± 0.09	1.6 ± 0.3	1.5 0.2

[a] *$p < 0.05$, **$p < 0.01$ (Student's paired *t*-test, no EPI *vs.* EPI, within the same group, at the same time).

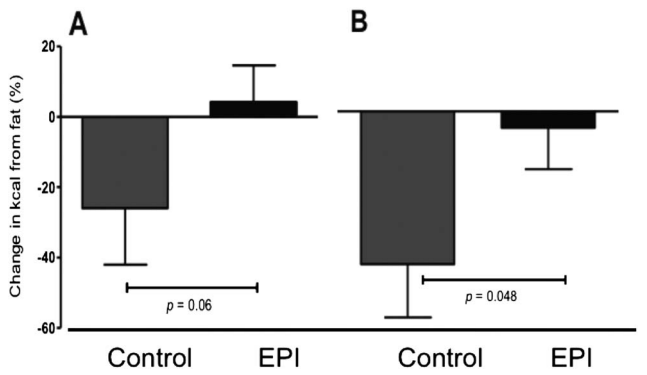

Fig. 3 Comparison of percent change in lipid oxidation, at 2 hours after meal ingestion (*vs.* the fasting state) between (A) normal ($n = 12$) and (B) overweight ($n = 8$) subjects. Non-parametric analyses were performed to compare differences between means. Postprandial fat oxidation in overweight subjects decreased more than among the normal subjects in the absence of EPI. However, with EPI supplementation such decrease was blunted.

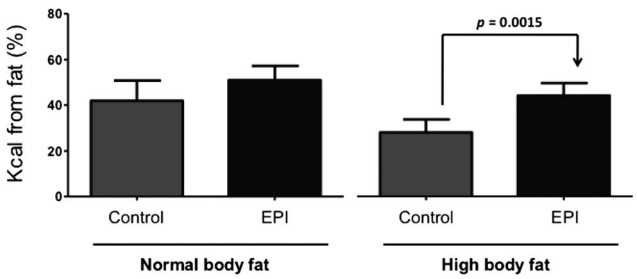

Fig. 4 Comparison of lipid oxidation between normal and high body fat subjects, 2 hours after meal ingestion. Lipid oxidation was significantly increased in high body fat subjects. Data are expressed as mean ± SD, *p* values by paired *t*-tests.

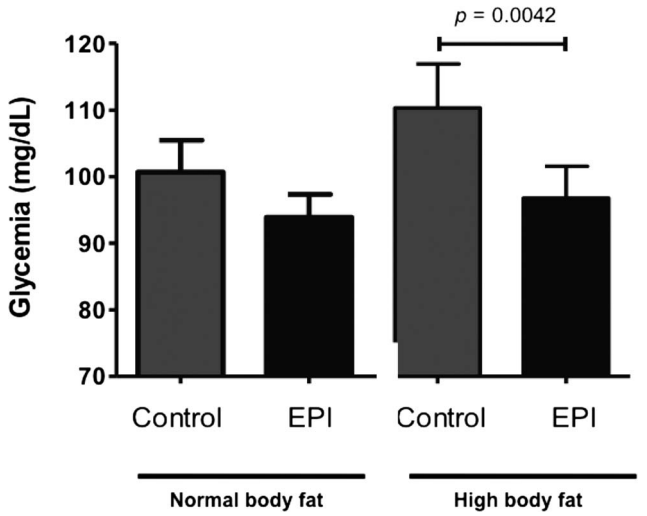

Fig. 5 Comparison of glycemia between normal and high adiposity subjects, 2 hours after meal ingestion. Glycemia was significantly attenuated in high adiposity subjects. Data are expressed as mean ± SD, *p* values by paired *t*-tests.

3.3 *Post-hoc* analysis by adiposity

Data were re-analyzed according to total body fat percentage cut-off points (men ≥25%, and women ≥32%),[13] yielding new groups: $n = 12$, normal body fat; $n = 8$, obese. As shown in Fig. 4, in subjects with normal fat mass, a non-significant increase in lipid oxidation at 2 h was noted, while in obese (*i.e.*, high adiposity) subjects a larger magnitude and significant effect were observed.

Fig. 5 shows that, in lean (normal body fat) subjects, EPI yielded a non-significant decrease in 2 h glycemia while it reached a significant reduction in obese (high body fat) subjects. Finally, as shown in Fig. 6, in both lean and obese subjects, EPI yielded significant decreases in 2 h triglyceridemia. Such an effect was more conspicuous (−12.3% and −16%) in subjects with excess body fat.

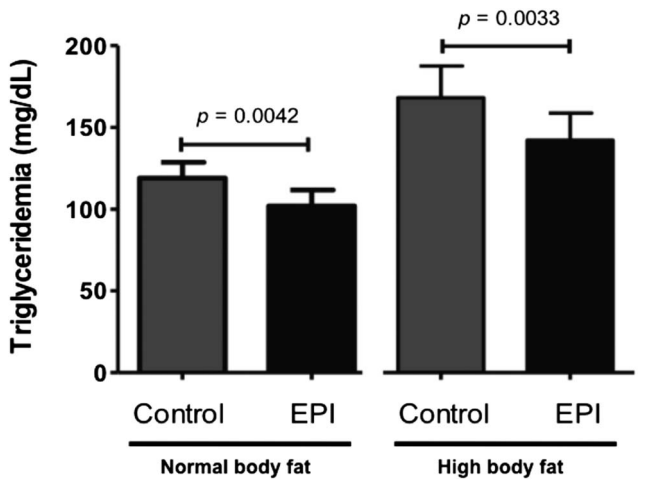

Fig. 6 Comparison of triglyceridemia between normal and high body fat subjects, 2 hours after meal ingestion. Triglyceridemia was attenuated in high adiposity subjects. Data are expressed as mean ± SD, *p* values by paired *t*-tests.

4. Discussion

Unique findings of this study are that EPI supplementation lowers the RQ ratio during the postprandium thereby reflecting increased fat oxidation. In addition, postprandial increases in glycemia and triglyceridemia are also attenuated by the flavanol. These results therefore suggest that EPI acutely modulates postprandial metabolism. To our knowledge, no other agent (neither nutraceutic nor pharmacologic) has been reported to exert such an effect after a single dose.

After food intake, glucose enters the portal blood stream and some of it bypasses the liver and reaches the systemic circulation, thus stimulating insulin release, which induces glucose uptake, mainly by skeletal muscle. As a result, glycemia increases transiently as glucose undergoes rapid metabolism and thus, it is cleared from the blood in a relatively short period (4 h, *i.e.*, the postabsorptive state). Dietary lipids are absorbed into lymphatic circulation as chylomicrons and, then, they enter the liver as remnants of chylomicrons, where a part of them are hydrolyzed by hepatic triglycerides lipase and used in the assembly of VLDL in the blood stream. Such fats are redistributed throughout the body for their oxidation (*e.g.*, in skeletal muscle) or storage (mainly in adipose tissue), thus decreasing lipemia in the late postprandium.

In accordance with energy substrate utilization, "normal" postprandial calorimetry should follow a pattern similar to that shown in Fig. 1. A healthy subject should exhibit a rise in RQ after consuming a standard meal, due to carbohydrate oxidation. Afterwards, the RQ should drop again as lipids are now the preferred energy fuel.

4.1 Reasoning behind this study

Although clinical blood chemistry diagnostic tests are currently performed in the fasting state, recent reports suggest that postprandial metabolic dysfunctions, such as hyperglycemia and hyperlipidemia, represent independent risk factors for the development of CV disease.[15] In fact, several studies have demonstrated that fed state blood glucose and triglycerides values are more strongly associated with all-cause CV morbidity and mortality.[16] The OMTT, besides allowing the study of postprandial metabolism, is a closer approach, than oral glucose tolerance tests, to the actual food ingestion as the nutritional supplement contains proteins, carbohydrate and fats, rather than pure glucose. Thus an OMTT-based postprandial metabolic profile reflects more closely what would the study subject present in real-life situations.

The normal subjects' OMTT patterns, from both blood determinations and indirect calorimetries, were similar to the theoretical curves illustrated in Fig. 1. A transient rise in glycemia and triglyceridemia was observed; however, mean values fell within the currently accepted cut-off points for postprandial blood glucose and triglycerides (*i.e.*, at 2 h, <7.7 mmol L^{-1} and <1.7 mmol L^{-1}, respectively).[17] Accordingly, during the postprandium, the RQ rose towards glucose catabolism and dropped back 4 h later.

In contrast, results in the overweight subjects suggest underlying metabolic imbalances: even though fasting blood glucose was not different from that of the normal-weight subjects, the overweight group did present borderline dyslipidemia at 0 h. Moreover, blood chemistry values were concordant with those of the calorimetric assessment as the RQ was higher than that of the normal-weight subjects. Taken together, these results reflect impaired lipid oxidation, which is a widely described metabolic alteration concomitant to excess weight and/or obesity. Other studies have reported that overweight subjects exhibit higher RQ's than their control counterparts.[18] Such metabolic perturbations contribute to elevated lipemia and may favor future insulin resistance and the development of type 2 diabetes and arterial hypertension (*i.e.* metabolic syndrome). During the OMTT, as expected, overweight subjects relied more on carbohydrate catabolism as they had a higher RQ than normal-weight subjects; these results correlated with steeper increases in postprandial blood glucose and triglycerides. These phenomena, again, evidence the underlying disturbances in lipid metabolism of overweight subjects, as reported in the literature.[19]

4.2 EPI effects on the OMTT

EPI attenuated postprandial hyperglycemia and hypertriglyceridemia, while enhancing fat oxidation, in both normal- and overweight groups, although the magnitude of the effect was greater among the latter.

Other polyphenols, especially epigallocatechin gallate (EGCG), the most abundant flavonol in green tea, have also been reported to regulate energy metabolism, through the capability of lowering the RQ, and decreasing plasma glucose and lipids.[20] In addition, other polyphenols, including anthocyanins, resveratrol and curcumin, have also been reported to lower triglyceridemia and glycemia.[21] The acute supplementation of such agents has been reported to result in similar findings to those of the present study: the lower after-meal RQ, decreased postprandial lipemia and improved glucose tolerance.[22] However, these investigations cannot be accurately compared due to their non-standardized polyphenols and/or carrier composition; most of them have been supplemented with either extracts (*e.g.*, green tea or pomegranate extract) or foods with high-polyphenol content. Hence it would be inaccurate to state that, specifically, such or such a substance is responsible for the observed changes.

A second issue in comparing our study with others is that the effects also depend on the dosage and the frequency of the compound supplementation. First, the majority of the studies report that improvements on metabolic profiles are achieved with much higher doses: 300 mg of EGCG,[23] 200 mg of caffeine,[12] 1 g of resveratrol,[24] or 100 mg kg^{-1} of quercetin.[25] Moreover, such studies report their metabolic improvements only after repeated dosing, implying that only long-term interventions can improve postprandial disturbances.[26] In contrast, our results show that EPI decreased blood lipids in a shorter period (30 minutes) and when given as a single dose.

Interestingly, the only therapy that closely mimics the effects of EPI herein found is exercise. Several reports have shown that 30 minutes – the same time for EPI – of moderate to vigorous physical activity result in major acute health benefits, including improvements in postprandial lipemia and glycemia, enhanced glucose uptake, and increased fat oxidation.[27]

In fact, molecular mechanisms that may be involved in EPI effects include the AMP-activated protein kinase (AMPK), which is activated through exercise performance. Polyphenols, such as EPI, have been proposed as potent activators of AMPK as they increase the enzyme's activity, resulting in improvements in the overall oxidative (*i.e.* metabolic) profile, through the mobilization of fat stores for lipid oxidation, activation of translocases and inhibition of lipogenic enzymes.[28] Our rodent studies provide evidence for such kind of effects upon EPI administration.[14]

In conclusion, EPI improves postprandial metabolism in healthy subjects, and to a greater extent in overweight subjects. These findings make EPI an interesting nutraceutical candidate since it mimics the effects of caloric restriction, exercise, and drugs like metformin. However, further vigorous and with bigger sample size investigations are necessary in order to confirm these preliminary findings and identify the underlying mechanisms.

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