Postprandial effects of dark chocolate on portal hypertension in patients with cirrhosis: results of a phase 2, double-blind, randomized controlled trial\textsuperscript{1–5}

Andrea De Gottardi, Annalisa Berzigotti, Susana Seijo, Mario D’Amico, Wolfgang Thommann, Juan G Abraldes, Juan Carlos García-Pagán, and Jaime Bosch

ABSTRACT

Background: In cirrhosis, hepatic endothelial dysfunction as a result of oxidative stress contributes to the postprandial increase in hepatic venous pressure gradient (HVPG).

Objective: We aimed at testing the hypothesis that dark chocolate, which holds potent antioxidant properties, might attenuate the postprandial increase in HVPG in patients with cirrhosis.

Design: In this phase 2, double-blind, controlled study, 22 cirrhotic patients referred for HVPG measurement were included and randomly assigned to receive a liquid meal containing either dark chocolate (active treatment; 85% cocoa, 0.55 g/kg body wt; n = 11) or isocaloric amounts of white chocolate (devoid of cocoa flavonoids; control subjects; n = 11). HVPG, arterial pressure, portal blood flow, serum flavonoids (catechin and epicatechin), and nitric oxide were measured at baseline and 30 min after meal administration. The main outcome measure was the change in HVPG 30 min after the test meal.

Results: Postprandial hyperemia was accompanied by a marked increase in HVPG in the white-chocolate group (16.0 ± 4.7–19.7 ± 4.1 mm Hg or +26.4 ± 12.7%; P < 0.0001), whereas the postprandial increase in HVPG was markedly attenuated in the dark-chocolate group (16.9 ± 2.9–18.7 ± 3.5 mm Hg or +11.5 ± 15.9%; \( P = 0.02 \) compared with white chocolate). Portal blood flow increased similarly after meals containing dark or white chocolate (median increase: 32% compared with 39%). Plasma flavonoids increased 15–50-fold after dark chocolate consumption. Dark but not white chocolate induced a mild increase in arterial pressure (+8.8 ± 8.8% compared with −0.3 ± 4.9%; \( P = 0.002 \)).

Conclusion: In patients with cirrhosis, dark chocolate blunted the postprandial increase in HVPG by improving flow-mediated hepatic vasorelaxation and ameliorated systemic hypotension. This trial was registered at clinicaltrials.gov as NCT01408966. \textit{Am J Clin Nutr} 2012;96:584–90.

INTRODUCTION

Increased hepatic resistance is the main determinant of portal hypertension in cirrhosis (1). Increased hepatic resistance is determined on one hand by the disturbance in liver vascular architecture (the so-called structural component) and on the other hand by increased hepatic vascular tone (2), because of the contraction of activated hepatic stellate cells, liver myofbroblasts, and vascular smooth muscle cells (3). This “dynamic” component accounts for as much as 30% of total liver resistance in cirrhosis and represents a novel target for therapy (4, 5). Hepatic endothelial dysfunction (6–8), a concept indicating the inability of the liver circulation to relax appropriately in response to increased blood flow, a result of decreased bioavailability of nitric oxide (NO)\textsuperscript{a}, prevents liver sinusoids from relaxing appropriately when blood flow increases and contributes to increase hepatic vascular tone. Oxidative stress worsens hepatic endothelial dysfunction and represents a target for drug therapy in cirrhosis (1).

In cirrhosis, because of endothelial dysfunction, the physiologic increase in splanchnic blood flow after the ingestion of a meal (postprandial hyperemia) cannot be accommodated by the liver circulation, resulting in a marked postprandial increase in portal pressure or its equivalent hepatic venous pressure gradient (HVPG), which is maximal 30 min after a test meal (9, 10).

Measurements of HVPG before and after administration of a test meal have been used in patients with cirrhosis to evaluate the efficacy of drugs modulating hepatic endothelial dysfunction (11–13). Low doses of NO donors, such as isosorbide-5-mononitrate (11), antioxidants (intravenous ascorbic acid) (12), and statins, which
increase endothelial NO synthase activity (13), have been shown to attenuate the postprandial increase in portal pressure in patients with cirrhosis by ameliorating hepatic endothelial dysfunction.

In recent years, increasing evidence has shown that dark chocolate, which contains a high proportion of antioxidant cocoa flavonoids (14), increases NO availability in the systemic circulation (15). The flavonoid epicatechin is causally linked to the beneficial vascular effects observed in humans after the ingestion of dark chocolate, which contains large amounts of flavonol-rich cocoa (16). In populations showing systemic endothelial dysfunction (17), cocoa acts as a potent antioxidant and improves endothelial function (18). Moreover, in heart-transplanted patients, dark chocolate has been shown to improve coronary vasomotion (19). Finally, recent meta-analyses confirmed the favorable cardiovascular profile of flavonol-rich cocoa products (20, 21).

Given these observations, we hypothesized that the antioxidant properties of dark chocolate could be beneficial in patients with cirrhosis, because they might improve intrahepatic endothelial dysfunction. Consequently, the aim of this study was to evaluate whether a dark chocolate-containing test meal may attenuate by ≥50% the postprandial increase of HVPG in patients with cirrhosis and portal hypertension.

SUBJECTS AND METHODS

Study design and protocol

We designed a proof-of-concept single-center, phase 2, double-blind, randomized controlled study. From September 2008 to December 2009, patients referred to our unit for hemodynamic evaluation of portal hypertension were considered for the study. Inclusion criteria were age >18 y, diagnosis of cirrhosis (proven by biopsy or clinical, laboratory, or imaging procedures), presence of esophageal varices of any grade (22), and HVPG ≥10 mm Hg during the hemodynamic study. Exclusion criteria were food allergy to chocolate, ongoing treatment with aspirin or other antioxidants, diffuse or multinodular hepatocellular carcinoma, pregnancy, advanced hepatic failure (defined as prothrombin ratio <40% and bilirubin >5 mg/dL), renal failure (defined by a serum creatinine concentration >1.5 mg/dL), portal vein thrombosis, cardiac or respiratory failure, and previous surgical or transjugular intrahepatic portosystemic shunt. The Consolidated Standards of Reporting Trials flow diagram of the study is shown elsewhere (see Supplementary Figure under “Supplemental data” in the online issue) (23).

The study protocol was approved by the Ethics Committee of the Hospital Clinic in May 2008 and was performed according to the principles of the Declaration of Helsinki (revision of Edinburgh 2000); the nature of the study was explained to the patients, and written informed consent was obtained in each case.

The primary outcome was the postprandial change in HVPG expressed both as percentage and absolute changes. Secondary outcomes were changes in portal blood flow, total hepatic blood flow, heart rate, systemic arterial pressure, nitric oxide metabolites (NOX), and flavonoids (catechin and epicatechin).

This study was performed on 2 consecutive days. On day 1, patients underwent baseline HVPG measurement and blood analysis. After completing the baseline measurements, the patients were randomly allocated (by a computer-generated random sequence; codes were contained in opaque sealed envelopes and opened by the research nurse; doctors were not aware of the code) to receive under double-blind conditions a liquid test meal (200 mL Ensure Plus; Abbott) complemented with either dark chocolate (rich in flavonoids) or white chocolate (devoid of flavonoids, used as controls).

Chocolate was administered as a milkshake consisting of semiskim milk (Llet Nostra Semidesnatada) with either 0.55 g dark chocolate/kg body wt (Lindt Excellence 85% Cocoa; Lindt & Sprüngli España) or 0.63 g white chocolate/kg body wt (Lindt Excellence Natural Vanilla; Lindt & Sprüngli España) in an isocaloric and isovolumetric proportion adjusted to body weight. The detailed macronutrient composition of the milkshake and Ensure Plus is reported elsewhere (see supplementary Tables 1–3 in the online issue). The total volume and calories of the milkshake were similar to those of standard test meals previously used in our laboratory and in studies assessing the meal-stimulated increase in HVPG (11–13). For a person weighing 70 kg, the content of the liquid meal corresponded to a piece of 85% cocoa dark chocolate of 38.5 g.

To allow a double-blind study, the milkshake was prepared by a person not involved in the study, placed in a nontransparent glass covered with a lid, and administered to the patient through a dark straw by a nurse. The physician performing the hemodynamic study was blinded to the milkshake composition. The meal was ingested over 8 min. Thirty minutes after meal ingestion, when maximal postprandial hyperemia and increase in HVPG have been demonstrated to occur (9–11), all of the measurements were repeated.

On day 2, patients underwent a baseline and postprandial noninvasive assessment of portal and hepatic artery blood flow by US Doppler. The test meal was prepared and administered after the same protocol used on day 1. The physician performing this second part of the study was blinded to both the composition of the milkshake and the results of the HVPG measurements.

Sample size calculation

Our previous studies in this field (11–13) showed that the postprandial increase in HVPG is reduced by ~50% by drugs able to reduce intrahepatic resistance; eg, in the article by Zafra et al (13), the postprandial increase in HVPG was 1.1 ± 1.3 mm Hg in patients treated with simvastatin compared with 2.8 ± 0.9 mm Hg in the control group. On the basis of these data, we hypothesized that there could be a 50% difference in the postprandial increase of HVPG between dark- and white-chocolate–treated patients. Considering an α risk = 0.05, a β risk = 0.20, an SD of 12%, and an analysis of the results by Fisher’s exact 2-tailed test, the sample size calculation was 22 patients (11 in each group).

HVPG, heart rate, and systemic arterial pressure

After fasting overnight, the patients were transferred the next morning to the Hepatic Hemodynamic Laboratory. Under local anesthesia, an 8F venous catheter introducer (Axcess, Maxxim Medical) was placed in the right jugular vein under ultrasonographic guidance (Sonosite Inc) by using Seldinger’s technique. Under fluoroscopic control, a 7F balloon-tipped catheter (Medi-
Portal blood flow and hepatic artery blood flow by US Doppler

Patients were studied by ultrasound by using last-generation duplex equipment (Sequoia 512; Acuson) with a multifrequency 4.5–7 MHz convex probe provided by a color, power, and pulsed Doppler device, after laying in a supine position for 10 min. Doppler measurements were obtained in triplicate by the same experienced operator during suspended normal respiration, and the results were expressed as their mean (25). Blood flow velocities were obtained by placing the sample in standardized positions of portal vein and hepatic artery (in the extrhepatic vessels, at the crossing between hepatic artery and portal vein), setting its dimension as wide as >50% of the vessel diameter and adjusting the insonation angle to a <60° position. Mean maximum velocity was automatically calculated after the operator had manually traced the border of Doppler waveform. To obtain mean velocity, the value was multiplied by 0.57 for venous vessels, and by 0.62 for arterial vessels (25).

Portal vein blood flow (PVBF) was derived from the following equation:

\[
PVBF \text{ (mL/min)} = \frac{\pi PV \text{ diameter (cm)}^2}{\Pi PV \text{ mean blood flow velocity (cm/s)} \times 60}.
\]

Similarly, hepatic artery blood flow (HABF) was derived from the following equation:

\[
HABF \text{ (mL/min)} = \frac{\pi HA \text{ diameter (cm)}^2}{\Pi HA \text{ mean blood flow velocity (cm/s)} \times 60}.
\]

Total hepatic blood flow was calculated as the sum of PVBF and HABF (26).

Biochemical measurements

In a subgroup of 18 patients (10 in the dark-chocolate group and 8 in the white-chocolate group), blood samples from a peripheral vein were collected at baseline and 30 min after the test liquid meal supplemented of either dark or white chocolate. Plasma was separated within 15 min and frozen at −70°C for subsequent analysis. An HPLC method, with the use of fluorescence detection, was adapted for the measurement of catechin and epicatechin in human plasma. Sample preparation was similar to that described by Rein et al (27). It involved an enzymatic deglucuronidation step with β-glucuronidase/arylsulfatase and precipitation of plasma proteins with methanol, followed by solid-phase adsorption of the compounds of interest to aluminum oxide. After being washed with water and methanol, bound catechin and epicatechin were detached with perchloric acid and analyzed by using 4-methylcatechol as internal standard. Separation was performed on an Atlantis dC18 column by using an eluent comprising a 78/22 (vol/vol) mixture of 50 mmol/L phosphate buffer (pH 4.0) and acetonitrile.

The excitation and emission wavelengths were set at 208 and 316 nm, respectively. HPLC allowed the separation and the identification of catechin and epicatechin with a linear calibration between 5 and 100 ng/mL. The lower limits of quantification for both compounds were 2.5 ng/mL and, for all concentration, interday relative SD values were <11% (n = 6). NOx from peripheral venous blood were measured by chemiluminescence (Nitric Oxide Analyzer, NOA 280; Sievers Instruments) in 7 patients per group.

Data analysis

Statistical analysis of the data was performed by using SPSS 15.0 (SPSS Inc). Continuous variables are expressed as means ± SDs (normal distribution) or as medians (ranges) (nonnormally distributed). Comparisons between baseline and postprandial values within groups were assessed by Wilcoxon tests for paired samples. Both absolute and percentage changes were assessed. Differences between the dark- and white-chocolate groups were evaluated by using the Mann-Whitney U test for continuous variables and by Fisher’s exact test for frequencies. Correlations were assessed by Spearman’s linear coefficient. Differences were considered statistically significant if 2-sided P values were <0.05.

RESULTS

From September 2008 to December 2009, 22 patients were randomly assigned and received either dark (n = 11) or white (n = 11) chocolate supplementation to the test meal. Clinical and hemodynamic baseline data of the patients are shown in Tables 1 and 2. No significant differences were found between the dark- and white-chocolate groups, except for a hazard-related male predominance in the dark-chocolate group.

Primary outcome

HVPG increased 30 min after the test meal from 16.0 ± 4.7 to 19.7 ± 4.1 mm Hg (P < 0.0001) in the white-chocolate group and from 16.9 ± 2.9 to 18.7 ± 3.5 mm Hg (P = 0.045) in the dark-chocolate group. However, the increase in HVPG in patients receiving dark chocolate was significantly less than the increase in patients receiving white chocolate. The respective increases were 1.8 ± 2.6 compared with 3.7 ± 1.1 mm Hg (P = 0.022) or 11.5 ± 15.9 compared with 26.4 ± 12.7% (P = 0.028).

Because men were more prevalent in the dark-chocolate group, we performed a linear backward regression analysis to exclude that the observed difference in postprandial HVPG was related to sex. Postprandial change in HVPG was entered into the analysis as a dependent variable, and sex and dark- and white-chocolate groups as predictors; sex was excluded from the final model (P =
0.777), whereas chocolate group remained significantly associated with HVPG change (P = 0.016).

Secondary outcomes

Portal blood flow, hepatic artery blood flow, and total hepatic blood flow by US Doppler

Portal vein blood flow (+229 ± 159 compared with +251 ± 261 mL/min; NS) and total hepatic blood flow (+181 ± 203 compared with +105 ± 295 mL/min; NS) increased significantly and similarly after the test meal in patients who received dark and white chocolate, respectively; hepatic artery blood flow decreased slightly and to a similar extent in both groups, respectively (-15.4 ± 55.1% compared with -15.4 ± 61.9%; NS).

Heart rate and systemic arterial blood pressure

Mean arterial pressure remained unchanged after the test meal in the white-chocolate group, whereas in the dark group it slightly increased (+7.1 ± 7.6 mm Hg, P = 0.01 compared with baseline). Heart rate did not change after the test meal in both groups.

Catechin, epicatechin, and NOx

Catechin and epicatechin did not change in patients who received a liquid meal containing white chocolate, remaining in all cases either undetectable or close to the quantification limit of the assay. These compounds markedly and significantly increased in patients who received dark chocolate supplementation (catechin, 10-fold increase; epicathechin, 50-fold increase) (Table 2).

NOx decreased significantly after the standard liquid meal supplemented with white chocolate (21.9 ± 16.5 compared with 24.5 ± 17.7 nmol/mL; P = 0.018), whereas it nonsignificantly increased in patients who received dark chocolate (21.7 ± 10.2 compared with 20.5 ± 11.3 nmol/mL; P = 0.31 compared with baseline; P = 0.013 compared with white chocolate) (Table 2).

DISCUSSION

Chocolate has been attributed beneficial properties on human health and mood since ancient times (28). It was not until recently, however, that scientific evidence showed convincingly that dark chocolate has definite beneficial effects on vascular...
function, including an improvement in vascular response in patients with conditions associated with endothelial dysfunction (20, 21, 29, 30). On the basis of these facts, we hypothesized that dark chocolate may have beneficial effects on portal hypertension caused by cirrhosis, because it has been shown that hepatic sinusoidal endothelial dysfunction contributes to increase the hepatic vascular tone (6, 7) and, by this mechanism, worsens portal hypertension in patients with cirrhosis (31).

The results of this proof-of-concept study showed that dark chocolate supplementation to a liquid meal had beneficial effects on portal hypertension in patients with cirrhosis, as shown by a marked attenuation of the postprandial increase in portal pressure. Patients who received a meal supplemented with dark chocolate showed less than half of the increase in HVPG observed in control patients who received a meal containing white chocolate (devoid of cocoa flavonoids). Interestingly, this beneficial effect was achieved despite a similar postprandial increase in portal blood flow or total hepatic blood flow. This suggests that dark chocolate acted favorably ameliorating the vasodilatory response of the liver circulation to postprandial hyperemia, decreasing the hepatic vascular tone and thus limiting the increase in portal pressure.

### TABLE 2

<table>
<thead>
<tr>
<th>Absolute change and percentage change in hemodynamic variables, NOx, catechin, and epicatechin after the standard meal supplemented with white or dark chocolate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White chocolate</strong></td>
</tr>
<tr>
<td><em>(n = 11)</em></td>
</tr>
<tr>
<td>HVPG</td>
</tr>
<tr>
<td>Baseline (mm Hg)</td>
</tr>
<tr>
<td>Postprandial (mm Hg)</td>
</tr>
<tr>
<td>Absolute change (mm Hg)</td>
</tr>
<tr>
<td>Percentage change</td>
</tr>
<tr>
<td>Portal blood flow</td>
</tr>
<tr>
<td>Baseline (mL/min)</td>
</tr>
<tr>
<td>Postprandial (mL/min)</td>
</tr>
<tr>
<td>Absolute change (mL/min)</td>
</tr>
<tr>
<td>Percentage change</td>
</tr>
<tr>
<td>Total hepatic blood flow</td>
</tr>
<tr>
<td>Baseline (mL/min)</td>
</tr>
<tr>
<td>Postprandial (mL/min)</td>
</tr>
<tr>
<td>Absolute change (mL/min)</td>
</tr>
<tr>
<td>Percentage change</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>Baseline (mm Hg)</td>
</tr>
<tr>
<td>Postprandial (mm Hg)</td>
</tr>
<tr>
<td>Absolute change (mm Hg)</td>
</tr>
<tr>
<td>Percentage change</td>
</tr>
<tr>
<td>Heart rate</td>
</tr>
<tr>
<td>Baseline (beats/min)</td>
</tr>
<tr>
<td>Postprandial (beats/min)</td>
</tr>
<tr>
<td>Absolute change (beats/min)</td>
</tr>
<tr>
<td>Percentage change</td>
</tr>
<tr>
<td>NOx*</td>
</tr>
<tr>
<td>Baseline (nmol/mL)</td>
</tr>
<tr>
<td>Postprandial (nmol/mL)</td>
</tr>
<tr>
<td>Change (nmol/mL)</td>
</tr>
<tr>
<td>Percentage change</td>
</tr>
<tr>
<td>Catechin (ng/mL)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Postprandial</td>
</tr>
<tr>
<td>Absolute change</td>
</tr>
<tr>
<td>Epicatechin (ng/mL)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Postprandial</td>
</tr>
<tr>
<td>Absolute change</td>
</tr>
</tbody>
</table>

1 HVPG, hepatic venous pressure gradient; NOx, nitric oxide metabolites.
2 Refers to the comparison between the white- and dark-chocolate groups. *P < 0.05* indicates statistical significance. Comparisons between baseline and postprandial values within groups were assessed by Wilcoxon tests for paired samples. Differences between the dark- and white-chocolate groups were evaluated by using the Mann-Whitney U test for continuous variables and by Fisher exact test for frequencies.
3 Mean ± SD (all such values).
4 Determined in 7 patients per group.
5 Determined in 8 patients in the white-chocolate group and in 10 patients in the dark-chocolate group.
6 Median; range in parentheses (all such values).
Dark chocolate contains a remarkably high number of compounds (flavonoids, theobromine, caffeine, and a mixture of amino acids and dipeptide derivatives) and exerts psychoactive and stimulating effects on the central nervous system (32), which might indirectly regulate systemic and splanchnic vasomotor responses. Given the complexity of dark-chocolate composition, we could not assess whether its hepatic hemodynamic effects were due only to catechin and epicatechin, which increased by 10–50-fold after the test meal only in patients who received dark chocolate and which were previously identified as responsible for some beneficial cardiovascular effects of dark chocolate (16). Whatever the exact molecules involved, in our study we documented a postprandial increase in oxidative stress (as assessed by dosing the circulating concentrations of NOx) only in patients who received white chocolate but not in those who received dark chocolate, which suggests an antioxidant effect analogous to that observed in studies involving patients with cardiovascular diseases (14–19). The antioxidant effects of dark chocolate are thought to be the main mechanism by which it improves systemic endothelial dysfunction in human diseases (14–19); we hereby demonstrate similar favorable effects in patients with cirrhosis occurring at the hepatic circulation, precisely where there is an abnormal increase in the vascular tone that contributes to raise portal pressure. This agrees with previous studies by our group in both experimental models and in patients with cirrhosis, which indicates that other antioxidants, such as superoxide dismutase (33), tetrahydrobiopterin (34), tempol (35), or vitamin C (12), ameliorate hepatic endothelial dysfunction.

We also found a slight improvement in arterial hypotension after dark, but not after white, chocolate consumption. The explanation for this finding remains speculative, but it is possible that the vasorelaxation of hepatic circulation might favorably influence the hyperdynamic syndrome associated with portal hypertension (1).

At least 2 limitations mitigate the positive findings of this study. First, there is no evidence that the blunted postprandial increase in HVPG in patients who received dark chocolate may be clinically relevant and associated with a better outcome. Second, the design of this study (one single administration of dark chocolate in a liquid meal) allows only the conclusion that the acute hemodynamic effects may be beneficial.

Hence, the addition to solid meals of a standard serving of dark chocolate—a tasty and fairly cheap product containing antioxidants devoid of unpleasant side effects—needs to be tested in appropriate long-term studies in cirrhotic patients.

Other limitations of the study include its small number of patients; however, the assumptions used to calculate the sample size were all met, so the power of the study was sufficient to verify our hypothesis. Even if the patients were randomly allocated to dark or white chocolate, some small differences because of chance were present at baseline; men were more prevalent in the dark-chocolate group; however, sex differences in HVPG response to a meal in patients with cirrhosis have never been reported.

Because this was a proof-of-concept study, we only examined what happened after a single meal; whether repeat long-term use is associated with similar, greater, or lower beneficial effects is unknown. In particular, the effects of the repeated addition of dark chocolate to meals on body weight should be carefully evaluated, because even though frequent chocolate consumption was recently shown to be linked to lower BMI (36) and to other metabolic benefits, including improved insulin sensitivity (37), weight gain resulting from increased caloric intake might have unfavorable consequences on the metabolic profile and prognosis in cirrhosis (38).

Moreover, the effects observed were modest in the sense that HVPG was not decreased below baseline values, so the probability that dark chocolate alone may be therapeutic is unlikely. Nevertheless, this study showed that the administration of dark chocolate could provide an additional beneficial effect on HVPG in patients who were already being treated with nonselective β-blockers. Larger studies investigating the clinical long-term effects of dark chocolate supplementation alone or in combination with β-blockers are required.

In conclusion, the results of this study suggest that dark-chocolate supplementation to a liquid meal exerts beneficial effects on hepatic hemodynamics in cirrhosis, attenuating the brisk postprandial increase in portal pressure and ameliorating systemic hypotension.

We thank Walter Angst (Lindt & Sprüngli, Spain) for providing the chocolate used in this study and A Baringo, MG Delgado, E Erice, A Flores, V La Mura, E Llop, S Rafia, L Rocabet, R Saez, S Seijo, R Vukotic, and R Theurillat for their help in carrying out this study.

The authors’ responsibilities were as follows—ADG, AB, and JB: designed the research (project conception, development of overall research plan, and study oversight); ADG, AB, MD, SS, JGA, and JCG-P: conducted the research (hands-on conduct of the experiments and data collection); WT: provided essential materials; ADG and AB: performed the statistical analysis and wrote the manuscript; and JB: had primary responsibility for the final content. All of the authors read and approved the final version of the manuscript. No conflicts of interest were declared.

REFERENCES

11. Bellis L, Berzigotti A, Abraïdes JG, Moïtinho E, García-Pagán JC, Bosch J, Rodés J. Low doses of isosorbide mononitrate attenuate the