Cholestatic liver injury induced by food additives, dietary supplements and parenteral nutrition

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ABSTRACT
Cholestasis refers to the accumulation of toxic levels of bile acids in the liver due to defective bile secretion. This pathological situation can be triggered by drugs, but also by ingredients contained in food, food supplements and parenteral nutrition. This paper provides an overview of the current knowledge on cholestatic injury associated with such ingredients, with particular emphasis on the underlying mechanisms of toxicity.

1. Introduction
Cholestasis describes any situation of impaired bile flow within the liver as such (intrahepatic cholestasis) or in the biliary tree (extrahepatic cholestasis) leading to the accumulation of toxic levels of bile acids (BAs) (Tanaka et al., 2017). In adult patients it is usually depicted as an increase in serum levels of alkaline phosphatase (ALP) to more than twice the upper limit of normal and an alanine aminotransferase (ALT) to ALP ratio (ALT/ALP) below 2 (Bjornsson and Jonasson, 2013). Additionally, although hyperbilirubinemia is not a synonym of cholestasis, cholestatic injury usually leads to impaired conjugated bilirubin excretion. Therefore, recent guidelines for establishing cholestasis limits its detection in in vitro models, which in most cases can only be used for short-term studies. A significant effort has been made in recent years to develop in vitro models that can better mimic the in vivo human scenario for longer periods of time (Gijbels et al., 2019; Vilas-Boas et al., 2019a). Spheroid cultures of primary human hepatocytes provide reliable and versatile tools for studying drug-induced cholestasis due to their similarity to the clinical situation and to the possibility of performing long-term studies (Baze et al., 2018; Bell et al., 2016; Hendriks et al., 2016).

The main mechanisms so far implicated in the onset of drug-induced cholestasis are summarized in Fig. 1. These include altered bile...
canalici dynamics, i.e. the constriction/dilation of the bile canalici due to activation/inhibition of the Rho kinase/myosin light chain kinase pathway (Burbank et al., 2016), and hepatocellular changes, such as cytoskeletal alterations and decreased membrane fluidity (Gijbels et al., 2019). Apart from these, the best described mechanism of drug-induced cholestasis relies on compromised activity of BA transporters. The most relevant BA transporters in this context are the bile salt export pump (BSEP) and the multidrug resistance-associated protein (MRP) 2, both exporters of BAs to the bile canaliculi, the MRP3 and MRP4 that export BAs to the sinusoidal blood, and the sodium-taurocholate co-transporting polypeptide (NTCP) involved in the uptake of BAs from the enterohepatic circulation (Yang et al., 2013).

The subsequent bile accumulation events evoke 2 types of cellular responses, namely an adverse response and an adaptive response. The adverse response is accompanied by the onset of processes, such as oxidative stress, inflammation and different cell death modes. The adaptive response, aimed at decreasing the uptake and increasing the export of BAs to and from hepatocytes, respectively, depends on the activation of several nuclear receptors (Gijbels et al., 2019).

Many chemical agents other than drugs, including industrial chemicals, cosmetic ingredients and biocides, have been related to cholestasis (Vilas-Boas et al., 2019b). This equally holds true for a number of chemical mixtures regularly used as food additives or common dietary supplements, such as herbal extracts. Amidst the main factors known to predispose individuals to dietary supplement-induced cholestatic liver injury are genetics, the concomitant use of drugs or other additives has been correlated with the development of cholestatic events in laboratory animals (Table 1). Among those are tartrazine and sunset yellow, which are used as colorants in food and cosmetics industry (Vilas-Boas et al., 2019b). EFSA has established an acceptable daily intake up to 7.5 mg/kg body weight (bw)/day for tartrazine and 4 mg/kg bw/day for sunset yellow (EFSA, 2009; 2014). In vitro studies have demonstrated that these compounds significantly activate human estrogen receptor alpha (ERα) transcriptional activity (Axon et al., 2012), which warrants their classification as xenoestrogens or, more broadly, endocrine disrupting chemicals. (Xeno)estrogens have been described to cause cholestasis through ERα-dependent decrease of transporter expression (Yamamoto et al., 2006) or ERα-stimulated transporter internalization (Barosso et al., 2012). More recently, the...
Polysorbate 80 has been approved, along with other polysorbates, as an emulsifier for foods and parenteral nutrition formulations, and as a solubilizing and dispersing agent for drug formulations and cosmetics. The acceptable daily intake was set at 10 mg/kg bw/day (EFSA, 2015b). Polysorbate 80 has been implicated in hepatic toxicity originating from the intake of vitamin E formulations, in which it was used as an emulsifier (Bhat et al., 1985). A subsequent study suggested its cholestatic potential in an isolated perfused rat liver model as evidenced by decreased bile flow, possibly due to increased membrane permeability (Ellis et al., 1996).

A recent study describes the systematic screening of EFSA safety evaluation reports to characterize different types of toxicity induced by food additives (Kramer et al., 2019). Clinical indicators of cholestasis were identified for iron tartrate, oxidized polyethylene wax and neotame, used as anti-caking agent, glazing agent and sweetener, respectively. These additives increased serum BAs and/or ALP levels in animal studies (Kramer et al., 2019).

### 3. Herbs and dietary supplements

Dietary supplements are considered a subset of foods that includes not only vitamins, minerals, amino acids and essential fatty acids, but also herbal extracts, usually in concentrated forms (EFSA, 2018a; FDA, 2018). They are popular agents that are perceived as natural and harmless, and do not require a prescription, which makes them easily accessible to the general public in an uncontrolled manner. The labeling legislation requires that the category or nature of the substances is described, along with some other predefined statements. However, the establishment of the product’s safety is the responsibility of the manufacturer, importer, supplier or distributor (EFSA, 2018a; FDA, 2018) and not of a public authority.

The intake of dietary supplements has been implicated as a causal factor in 2-15.5% of all reported cases of hepatotoxicity (Navarro et al., 2017; Stickel et al., 2011; Stickel and Shouval, 2015). These numbers may be slightly higher when considering the underreported events and the difficult diagnosis which, like for DILI cases, relies on the exclusion of every other possible cause(s) for the observed adverse effects (Tajiri and Shimizu, 2008). In most situations, withdrawal of the insult leads to recovery. The gold standard to establish causality is the rechallenge test, which is ethically debatable and clinically not recommended (Tajiri and Shimizu, 2008). Hepatotoxic herbs and dietary supplements are various in nature (Table 2). The complex composition of such supplements offers an additional challenge for the identification of the real causative agent. In general, genetic polymorphisms in cytochrome P450 (CYP) enzymes or in the immune response, the presence of underlying disease and the concomitant intake of other supplements or medication may predispose certain people to such type of hepatotoxicity (Elinav et al., 2007; Stolz et al., 2019).

#### 3.1. Herbal supplements

In the long list of plants known as germanders, the species *Teucrium polium* and *Teucrium chamaedrys* have been associated with liver injury, frequently with a cholestatic signature. They have been used as...
supplements for the control and management of weight, diabetes and hyperlipidemia (NIH, 2012). The mechanism of liver toxicity described so far relies on metabolic activation of the furano neoclerodane diterpenoids teucrin A and teuchmaedryn A to highly reactive epoxides inducers of hepatic apoptosis (Kotsiou and Christine, 2017).

Kava extract (Piper methysticum) has been used as part of herbal remedies to treat anxiety and insomnia, as the kavapyrones in the extract act as agonists of the gamma-aminobutyric acid receptor (Garcia-Cortes et al., 2008). Despite its traditional use in Polynesia as a beverage, kava has been withdrawn from many consumer markets in European countries (EMA, 2017) and has not been accepted in the US Pharmacopeia (NIH, 2012) due to liver injury concerns. The lactones composing the kava extract seem to be responsible for the inhibition of certain CYP enzymes as well as for glutathione depletion (Brown, 2017), though the results from preclinical and human studies are conflicting (NIH, 2012). The direct or indirect activation of Kupffer cells, and consequent release of inflammatory mediators and reactive oxygen species results in cholestatic inflammation (Rowe et al., 2011). The aqueous kava extracts, however, seem to be less hepatotoxic than the organic ones (Stickel and Shouval, 2015), although hepatotoxicity has been observed in both circumstances (Sarris et al., 2011). Both germander and kava stand out in the midst of the herbs with more related publications, though not case reports (Brown, 2017). These herbs are no longer sold in some countries but may still be available via the internet.

Polygonum multiflorum, re-classified as Reynoutria multiflora, is also known as Sho-Wu-Pian in China and as Fo-Ti in the US. It has for a long time been used in traditional medicine for its anti-aging properties, but other biological activities have been described, such as anti-inflammatory and anti-hyperlipidemic, among others. Stilbenes and anthraquinones are recognized as major characteristic constituents of this herb, but the real composition depends on whether it is used raw or processed, as this generates changes in the chemical composition (Bouda and Feng, 2015). Even though the most commonly observed hepatic injury follows a hepatocellular pattern (NIH, 2012), it has been implicated in a case of cholestatic hepatitis, which resolved after discontinuation (Park et al., 2001). Additionally, it was identified as possible cause of 25 incidents of toxin-induced acute hepatitis, 7 of which displayed a mixed hepatocellular and cholestatic pattern (Jung et al., 2011). In that report, the causality was probable because the herb was consumed in its natural form and most patients recovered after conservative measures and discontinuation of the insult. There was one positive rechallenge, which established the cause as definite. The mechanism of Fo-Ti-induced cholestasis is not known, yet it has been described as idiosyncratic (Jung et al., 2011). Increased levels of CYP7A1, the main enzyme involved in BA synthesis, were found in mice treated with 2,3,5,4-tetrahydroxystilbene-2-O-β-D-glucoside, another constituent of Fo-Ti (Chen et al., 2018), an effect that, in the authors’ opinion, could underlie the development of cholestasis after Fo-Ti ingestion. Interestingly, it was recently pointed out that the geographical origin of the herb can significantly impact the observed toxicity, possibly due to different composition of the analyzed preparations (Lin et al., 2017). This work also highlighted that even though emodin might be the most toxic component of Fo-Ti, it is not one of the main constituents and, therefore, most likely not the source of the hepatotoxicity. The same study identified tetrahydroxystilbene-O-(gallolyl)-hex and emodin-O-hex-sulfate as the possible toxic ingredients (Lin et al., 2017).

### 3.2. Bodybuilding and weight loss supplements

Hydroxycut® is a brand marketing products for weight loss and bodybuilding, which complex composition has been updated with time following records of cardiotoxicity and hepatotoxicity, including one fatal casualty (Fong et al., 2010). Hepatocellular injury is more frequently described, but a cholestatic pattern has been observed in some cases (Dara et al., 2008; Fong et al., 2010; Stevens et al., 2005). The initial formulation contained Ephedra, which was banned in 2004 (NIH, 2012). More recently, green tea (Camellia sinensis) extract, Garcinia cambogia and chromium have all been identified as possible contributors to the hepatotoxic effects (Dara et al., 2008). Hepatotoxicity has been noted after prolonged consumption of green tea extract, presenting a cholestatic or mixed hepatocellular and cholestatic signature in more than one third of the cases (Mazzanti et al., 2009). Considering the positive dechallenge and the positive rechallenge in 85% and in 20% of them, respectively, the causality was considered certain or definite (Mazzanti et al., 2009). Green tea has been widely consumed due to its alleged anti-cancer, anti-obesity, anti-diabetes, anti-inflammatory and neuroprotective effects (Miyoshi et al., 2015). Amidst
the distinct components, the catechin (−)-epigallocatechin gallate has the strongest bioactivity, but is also one of the main suspects, along with its metabolites, for the observed oxidative stress that results in hepatotoxicity (Stickel et al., 2011).

Other bodybuilding supplements have been associated with cholestatic manifestations, yet in many instances, a combination of dietary supplements was used, which makes it impractical to determine the causative agent. In this respect, Avelar and co-workers outlined the simultaneous use of NO-Xplode®, Growth Factor ATN® and SimiCarminita® by a 17-year old male presenting an acute mixed hepatocellular and cholestatic pattern of hepatotoxicity and acute renal failure (Avelar-Escobar et al., 2012). These supplements are all based on amino acids, resulting in excessive dosing when taken simultaneously. After treatment with cholestyramine, ursodeoxycholic acid and discontinuation of the supplements, biochemical parameters returned to normal within a month (Avelar-Escobar et al., 2012). A cholestatic type of liver injury has also been depicted in a 28-year old male following the combined use of NO-Xplode® and Hydroxycut® for 4 weeks (Peterson et al., 2013). The situation was normalized 4 months after the discontinuation of the supplements, and causality was determined as possible. A combination of creatine and whey protein supplements has been related to an occurrence of acute cholestatic liver injury with elevated bilirubin and ALP levels and a liver biopsy showing marked cholestasis (Whitt et al., 2008). The administration of intravenous fluids along with the discontinuation of the supplements led to recovery which, considering that other possible causes were excluded, correlates the episode with the intake of the supplements (Whitt et al., 2008). The weight loss supplement OxyElite Pro® has been associated with a number of cases of hepatotoxicity (Heidemann et al., 2016). One of the reported cases, concerns a 31-year old woman who took the supplement for 11 weeks before she was hospitalized. Liver biopsy revealed acute hepatitis with cholestasis. The absence of autoantibodies reaction excluded the possibility of an auto-immune hepatitis and OxyElite Pro® was described as the probable cause of the hepatotoxicity.

There have been many reports associating the intake of Herbalife™ supplements with the occurrence of acute liver injury, namely in Israel (Elinav et al., 2007), Switzerland (Schoepfer et al., 2007) and Spain (Manso et al., 2011). On the basis of the biochemical parameters, most of the cases were classified as hepatocellular injury, but liver biopsies revealed cholestasis insulits in a considerable number of them (Stickel and Shouval, 2015). Herbalife™ products are complex mixtures composed of plants and herbs supplemented with nutrients, commonly with an undisclosed composition, and consumers typically use multiple formulations simultaneously (Schoepfer et al., 2007). Clearly, this hinders the identification of the exact ingredient(s) causing the hepatotoxic effects. In this regard, hepatotoxicity caused by green tea extract cannot be ruled out as well as the hepatotoxic effects likely due to Aloe vera (Aloe barbadensis) (NIH, 2012). To date, the mechanism by which Herbalife™ products cause hepatotoxic injury, particularly with a cholestatic pattern, has not been elucidated, but the abundant immune cell infiltrates and the presence of auto-antibodies in some patients suggests an immune-mediated response (Elinav et al., 2007). Another ingredient that may be to blame for the cholestatic type of hepatotoxicity in Herbalife™ products is vitamin A, a regulator of BA homeostasis. Although vitamin A has been described to relieve extrahepatic cholestasis through the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) in a bile-duct ligation mouse model of cholestasis (Wang et al., 2014), its consumption in high doses has conversely resulted in increased basal levels of Nrf2 and decreased levels of transcripts involved in BA biosynthesis, such as CYP7A1, and BA transport, such as BSEP and NTCP in vivo (Lu et al., 2013). Additionally, oleanolic acid is an activator of the G protein-coupled BA receptor 1 (GPBAR1), also known as Takeda G protein-coupled receptor 5 (TGR5), which is a BA sensor involved in the maintenance of BA homeostasis. However, activation of TGR5 has shown to increase the expression of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in immune cells in vitro (Lou et al., 2014), creating a pro-inflammatory environment that contributes to the disturbance of BA homeostasis resulting in cholestasis (Woolbright and Jaeschke, 2019).

3.3. Hepatoprotective supplements

Some of the dietary supplements with the potential to promote liver injury are paradoxically marketed as hepatoprotective agents, namely celandine (Chelidonium majus), freshwater clam extracts and oleanolic acid. In particular, celandine has been used for treating gallstone disease, dyspepsia and irritable bowel syndrome (Benninger et al., 1999; Brown, 2017; Kotsiou and Christine, 2017). Its consumption underlies a number of cases of acute cholestatic hepatitis and, although not yet mechanistically understood, induced auto-immunity might play a role (Stickel et al., 2003). Causality has been confirmed by herbal withdrawal and rechallenge (Benninger et al., 1999; Stickel et al., 2003). Despite the well-established hepatotoxic episodes, the exact culprit(s) has(have) not been identified within the 20 possible molecules, including various biochemically active isooquinoline alkaloids (Kotsiou and Christine, 2017). The hepatoprotective properties of freshwater clam extract have been mentioned in ancient books and have been confirmed in rats (Hsu et al., 2010; Laurent et al., 2013). However, a recent study has correlated its consumption with cholestatic liver damage in humans, which was confirmed by the strongly positive results of the drug-induced lymphocyte stimulation test (Yokomori et al., 2016). Oleanolic acid is a natural pentacyclic triterpenoid found in many fruits, vegetables and herbs. Low concentrations of oleanolic acid have been found to protect the liver against lithocholic acid-induced toxicity in mice, likely due to its ability to activate Nrf2, which leads to increased expression of MRP2, MRP3 and MRP4 (Chen et al., 2014). However, prolonged exposure to increased doses of oleanolic acid resulted in cholestasis (Lu et al., 2013). Interestingly, this correlated with increased basal levels of Nrf2 and decreased levels of transcripts involved in BA biosynthesis, such as CYP7A1, and BA transport, such as BSEP and NTCP in vivo (Lu et al., 2013). Additionally, oleanolic acid is an activator of the G protein-coupled BA receptor 1 (GPBAR1), also known as Takeda G protein-coupled receptor 5 (TGR5), which is a BA sensor involved in the maintenance of BA homeostasis. However, activation of TGR5 has shown to increase the expression of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in immune cells in vitro (Lou et al., 2014), creating a pro-inflammatory environment that contributes to the disturbance of BA homeostasis resulting in cholestasis (Woolbright and Jaeschke, 2019).

3.4. Other herbal and dietary supplements

Other dietary supplements have only limited data on their cholestatic potential. Thus, Fructus psoraleae, the dried mature seeds of Psoralea corylifolia used to alleviate bone disorders and skin conditions, such as osteoporosis and psoriasis, respectively, has been implicated in a case of acute cholestatic hepatitis, albeit most likely due to an excessive intake (Nam et al., 2005). The chemical components of the seeds include psoralen, psoralidin, isobavachalcone, bakuchiol among others. Increased levels of CYP7A1, BSEP, MRP2 and MRP3 and decreased quantities of FXR have been found in female rats exposed to an ethanolic extract of the seeds for 28 days (Wang et al., 2012). The cholestatic effect was believed to involve the destruction of the biosynthesis and transportation of BAs, yet the specific causative component has not been identified. While a correlation between the increased CYP7A1 levels and the decreased FXR levels has been established (Wang et al., 2012), the increased levels of BA transporters could be a compensatory reaction, considering the prolonged exposure time. Of note, male rats displayed a distinct response, namely decreased amounts of FXR and CYP7A1 with no changes in BA transporter levels (Wang et al., 2012). This suggests gender-specific differences in sensitivity to the hepatotoxic effects of the extract. Venoplant® is composed of flavonoids extracted from Aesculus hippocastanum, used for the
4. Parenteral nutrition-associated cholestasis

The intravenous administration of nutrients, named parenteral nutrition (PN), is a beneficial way to provide nutrients wherever the enteral route is compromised or is insufficient to cover caloric and protein needs. Parenteral nutrition can be classified as total or partial, when patients only get nutrition through this route, or when nutrients are also provided via enteral route, respectively (Heidegger et al., 2013). However, parenteral nutrition is not without flaws. It may lead to many complications, including liver disorders, in particular, cholestasis (Beath and Kelly, 2016; Guglielmi et al., 2008). This is especially frequent during prolonged and/or total PN and in children (Fawaz et al., 2017; Jolin-Dahel et al., 2013), varying between 40 and 60% in infants and up to 85% in neonates under total PN (Lauriti et al., 2014).

PN-associated cholestasis (PN-AC) can be related to the amount, quality and duration of the nutrient infusion, enteral fasting and prematurity (Table 3). Premature children’s impaired immunity and immaturity of the enterohepatic circulation seem to prompt them more prone to developing cholestasis, particularly following total PN (Guglielmi et al., 2008). Those factors favor intestinal bacterial overgrowth, consequently increasing the deconjugation of chenodeoxycholic acid to the toxic lithocholic acid, as well as enteral fasting-induced enterocyte hypoplasia that facilitates bacterial translocation and the release of endotoxins (Kumar and Teckman, 2015). Endotoxins, such as lipopolysaccharides, and certain inflammatory mediators, such as TNF-α and cytokines, including IL-1β, are known downregulators of BA transporters, namely NTCP, BSEP and MRP2, contributing to the development of cholestasis (Kumar and Teckman, 2014). Furthermore, human fetal liver expresses lower levels of BA transporters than the adult liver (Chen et al., 2005), contributing for increased incidence of PN-AC in premature infants.

In the intestine, BAs act as natural activators of FXR, which in turn stimulates the production of FGF19. This growth factor is subsequently carried via portal circulation to the liver, where it binds the FGF receptor 4 (FGFR4), suppressing the expression of CYP7A1, also known as cholesterol 7α-hydroxylase, a major enzyme in BA synthesis from cholesterol (Fig. 2).

In the absence of BA release in the intestine due to enteral fasting, the FXR pathway is repressed, culminating in increased BA synthesis (Reinhner et al., 1989). The reduced levels of cholecytokinin observed during enteral starvation (Mashako et al., 1991) could also contribute for the onset of cholestasis, as this hormone stimulates the emptying of the gallbladder and reduces biliary sludge (Sitzmann et al., 1990). Controversially, animal studies revealed that the administration of cholecytokinin does not have an impact on the incidence of total PN-AC (Moss and Amii, 1999).

### Table 3

Main factors and suspected mechanisms of parenteral nutrition-associated cholestasis.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Mechanisms of hepatotoxicity</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Prematurity:</strong></td>
<td>- Overproduction/accumulation of toxic BAs</td>
<td>Guglielmi et al., 2008; Kumar and Teckman, 2014</td>
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<td></td>
<td>- Endotoxin-induced transporter inhibition</td>
<td>(Kumar and Teckman, 2015)</td>
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<tr>
<td><strong>Enteral fasting:</strong></td>
<td>- Facilitated bacterial translocation</td>
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<tr>
<td></td>
<td>- Enterocyte hypoplasia</td>
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<tr>
<td></td>
<td>- Absence of BA release</td>
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<tr>
<td><strong>Nutrients:</strong></td>
<td>- Protein, glucose</td>
<td>Gupta et al., 2016; Vileisis et al., 1980</td>
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<td></td>
<td>- Omega-6 fatty acids</td>
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<tr>
<td></td>
<td>- Phytosterols</td>
<td>Carter et al., 2007; El Kasmi et al., 2013</td>
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<tr>
<td></td>
<td>- Manganese</td>
<td>Carter et al., 2007</td>
</tr>
<tr>
<td></td>
<td>- Aluminum</td>
<td>Arnold et al., 2003; Klein et al., 1988</td>
</tr>
</tbody>
</table>

BA – Bile acid; FXR – Farnesoid X receptor; LXR – Liver X receptor; BSEP – Bile salt export pump; MRP2 – Multidrug resistance-associated protein 2.
The occurrence of PN-AC also seems to correlate with the amount and the quality of the infused nutrients, although the exact culprit and mechanisms have not yet been unveiled. Early reports have linked increased protein intake with a faster onset and increased magnitude of cholestatic jaundice (Vileisis et al., 1980). When comparing similar caloric intake, a glucose-rich regime seemed to disturb liver functions more than a high fat counterpart (Smirniotis et al., 1990). Subsequent retrospective studies assessing the role of each individual PN macronutrient, in casu dextrose versus protein versus lipids, correlated the daily intake of parenteral dextrose with a higher incidence of PN-AC (Gupta et al., 2016). The administration of a PN solution based on the amino acid composition of the umbilical cord was recently published not to differ from the breast milk-based one in what concerns the incidence of PN-AC (Anaya-Florez et al., 2019). In the past few years, focus has been put on the involvement of lipid emulsions in the induction of cholestasis (El Kasmi et al., 2013; Xu et al., 2012). The lipid component of PN may be either from vegetable or from animal origin. Soy oil-based lipid emulsions, the only FDA-approved lipid emulsions for children in the US, are rich in phytosterols, such as stigmastanol, and omega-6 fatty acids, like arachidonic acid (Amusquivar et al., 2008), known to act in a pro-inflammatory way. In particular, phytosterols antagonize FXR and the liver X receptor (LXR) therefore leading to activation of macrophages, downregulation of BA and bilirubin transporters, and interference with the synthesis and metabolism of BAs. These events ultimately result in cholestasis in vitro (Carter et al., 2007) and in vivo (El Kasmi et al., 2013). On the other hand, fish oil-based lipid emulsions, such as OmegavenTM, are mostly used in European countries. They have lower levels of omega-6 fatty acids and phytosterols, and increased amounts of essential omega-3 fatty acids, namely eicosapentaenoic acid and docosahexaenoic acid (Amusquivar et al., 2008). The anti-inflammatory activity of omega-3 fatty acids contributes to decreased triglyceride synthesis, decreased production of reactive oxygen species and other pro-inflammatory molecules in vitro, in animals and in humans (Kalish et al., 2013; Ventro et al., 2016). Ultimately, the inclusion of fish oil-based lipid emulsions, either alone or in combination with soy and olive oil-based ones, seems to exert a hepatoprotective effect, correlating with lower incidence or faster resolution of PN-AC, shorter hospitalization period, and reversal of intestinal failure-associated liver disease in infants (Calkins et al., 2014; Kasirer et al., 2019; Lee et al., 2016). Nowadays, most commercially available ready-to-use PN products contain a mixture of different fatty acids including soy bean, olive oil, medium chain triglycerides and fish oils to benefit from different characteristics and spread risks (Cai et al., 2018). Recently, it has been published that enteral fish oil supplementation, whenever possible, is an alternative to the more expensive intravenous administration, showing beneficial results, such as decreased duration of cholestasis and increased weight gain in infants (Thavamani et al., 2019). This and other studies support a beneficial role for the enteral administration of nutrients (Manzanares et al., 2015).

Micronutrients present in PN formulations, in particular manganese, have also been implicated in the development of cholestasis. High levels of manganese have been found in the blood of patients with PN-AC (Fell et al., 1996; Hambidge et al., 1989). Recommended intake levels for children ranged from 2 to 10 μg/kg/day in 1979, and decreased to 1 μg/kg/day as a consequence of such studies (Fell et al., 1996; Livingstone, 2018). In adults, this change was even more drastic, namely from 150 to 800 μg/day to 55 μg/day since 2014 (Livingstone, 2018). In adults, this change was even more drastic, namely from 150 to 800 μg/day to 55 μg/day since 2014 (Livingstone, 2018). However, it is still not clear whether hypermanganesemia causes liver disease or vice versa, as manganese is almost totally excreted via the bile (Livingstone, 2018). In many instances, manganese is only present as a trace contaminant of PN, which potentiates its accumulation and consequent toxicity, particularly in long-term schemes and patients with impaired biliary secretion (Livingstone, 2018). PN-AC has also been related to increased exposure to aluminum, also commonly found as contaminant. Most studies detected increased amounts of aluminum in infants under PN (Arnold et al., 2003; Fortenberry et al., 2017; Kumar and Teckman, 2015). Similarly, in vivo studies have shown that increased aluminum intake coexists with increased serum...
in vitro constraints and limitations, future research will fully rely on human liver-cancerous cell models that appropriately mimic the situation. In this context, spheroid cultures of primary human hepatocytes offer an excellent tool for long-term mechanistic studies of the cholestatic potential of drugs (Hendriks et al., 2016) and other chemicals.

In summary, recognizing potentially harmful food-based products and identifying the responsible ingredient(s) is one of the greatest challenges for correctly diagnosing and appropriately managing cholestatic liver injury due to the intake of food ingredients. The complex nature of most products, along with the simultaneous intake of interacting supplements or drugs, and the delayed onset of cholestatic damage, hampers the determination of causality. Therefore, the mechanisms by which cholestasis is triggered by these chemicals remain mostly unexplored. This knowledge is crucial for the improvement of the currently low clinical predictability of this type of chemical-induced liver injury, with potential impact on the actual regulation and, consequently, in overall human safety.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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