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Experimental Brain Death Models in Liver Transplantation

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http://dx.doi.org/10.5772/intechopen.75438

Abstract

Most organs for transplantation are currently procured from brain-dead donors; however, brain death is an important risk factor in liver transplantation. In addition, to counteract the shortage of liver grafts, transplant centers accept the use of sub-optimal livers, which may show higher risk of primary non-function or initial poor function. Very few literatures exist regarding liver transplantation using brain-dead donors, or about brain death and its effects on sub-optimal grafts in such surgical situation. This chapter aims to describe the pathophysiological changes occurring in liver grafts during brain death and focuses on the strengths and limitations of experimental models used to study the effect of brain death on optimal and sub-optimal (specially steatotic) liver grafts. Depending on the use of experimental models that simulate as much as possible the surgical conditions present in clinical practice, therapeutic strategies designed in animal models could be more successfully translated to the bedside.

Keywords: liver transplantation, ischemia-reperfusion injury, brain death, experimental models

1. Introduction

Liver transplantation (LT) has evolved to become a standard therapy for certain end-stage liver diseases [1]. Nowadays, 80% of organs come from donors who have suffered brain trauma (brain-dead donors) [1–4]. Brain death (BD) has been defined as the irreversible loss...
of brain and brain stem function, usually caused by major hemorrhage, hypoxia or metabolic dysregulation [5, 6]. Hemodynamic events, hormonal changes and inflammation and immune activation occur consequently to BD [6–9]. It has been described that BD markedly reduces the tolerance of liver grafts to preservation and reperfusion injury and reduces graft survival [2–4, 10]. The detrimental consequences of BD have been clinically described; however the underlying mechanisms and their relevance in LT remain poorly understood. Indeed, few studies have evaluated the effect of BD on LT, and most of the experimental studies focused in establishing surgical or pharmacological strategies to reduce liver graft damage associated with transplantation have been performed in the absence of BD [6].

Shortage of donor organs remains a major obstacle to the widespread application of LT in patients with end-stage liver disease [11–14]. To overcome this problem, transplant centers developed strategies to expand the organ donor pool [15]. This shortage could be alleviated by routine use of sub-optimal donor livers including elderly donors, steatotic donors, split livers and donor with viral infections or with malignancy. These sub-optimal livers exhibit a greater risk of organ dysfunction and primary non-function compared with optimal livers, when used as grafts in transplantation [1, 4, 9]. Multiple methods are currently being investigated to minimize the effects of ischemia-reperfusion (I/R) injury to allow the use of sub-optimal liver grafts, including anti-inflammatory approaches to attenuate cytokines, blockade of adhesion molecules, antiapoptotic strategies, among others. However, these studies are performed in non-BD surgical conditions. Only a recent study describes, for the first time, a potential treatment in steatotic liver grafts undergoing LT from cadaveric donors [1].

Since consequences of BD are linked to an inferior graft function and a more potent immune response, studies in animal models could be critical in uncovering its basic mechanisms and to provide a rationale for the development of novel targets that may prevent the deteriorating consequences of BD in clinical practice [16]. Virtually, all experimental organ transplantation studies generally utilize young, healthy living animals as donors; in clinical practice; however, a relatively low percentage of organs are acquired from living sources. Among other variables, the difference between living and brain-dead donors includes the effect of profound physiological and structural derangements [17]. Although a wide variety of animal BD models have been used to study the effect of BD on donor organ quality (without transplantation) [18–26], the lack of standardization of BD models makes difficult to compare results from different research groups. Undoubtedly, there is a need for a more controlled and standardized BD model allowing studies of organs in a situation closely resembling the reality in the intensive care unit [27].

In the first part of this chapter, we highlight the actual knowledge and new insights into the pathophysiology in BD focusing on liver graft undergoing transplantation. Following, the different experimental models reported in literature used to study the effect of BD on quality of optimal and sub-optimal liver grafts are presented, focusing on the strengths and limitations. Since recognize the underlying mechanisms of detrimental effects of BD is a critical question to design therapeutic strategies to protect liver grafts undergoing transplantation, we discuss if the existing BD animal models mimic the clinical findings in transplants with BD donors, and highlight which BD model could be more appropriated.
2. Effects of brain death on liver graft undergoing transplantation

Liver I/R injury is a local proinflammatory response mediated by the immune system. The central nervous system plays a fundamental role in the regulation of molecular markers triggering inflammation and tissue damage, and BD results in a breakdown of these mechanisms, hence initiating the cascade of I/R injury liver transplants and exacerbating the hepatic damage caused by I/R [9]. However, although the detrimental consequences of BD have been clinically described, the underlying mechanisms and their relevance in LT remain poorly understood [6]. Following, systemic events that happen after BD and the specific effects of BD on hepatic graft undergoing transplantation are presented.

BD is followed immediately by an acute and transient rise in blood pressure (Cushing reflex), that is immediately followed by a transient bradycardia communicated through parasympathetic activation [16, 24, 28–31]. Later after BD, deteriorating hemodynamics and a compromised perfusion of abdominal organs is becoming evident. Accordingly, a shift from aerobic to anaerobic metabolism and acidosis is registered, clinically reflected by elevated serum levels of lactate and free fatty acids and promoted by decreasing insulin secretion and hyperglycemia [21, 32, 33]. Alterations of specific mitochondrial functions may lead to an impaired production of ATP and a reduced uptake of substrates for mitochondrial metabolism and eventually limit resistance and survival of cells and organs to damaging insults [34]. It is well known that ATP degradation during hepatic ischemia leads to an acceleration of glycolysis [35]. Although glycolysis is essential for cell survival, it may also be detrimental because of the accumulation of glycolytic products such as lactate [35]. In the liver, the increase in cAMP levels due to ischemia triggers the activation of glycolysis. This causes the accumulation of hexose 6-phosphates, which proceed down the glycolytic pathway to form lactate [35–38]. Some authors [39] have shown that normotensive BD had no influence on the general viability of the liver, as measured by ATP content. In this research work, no differences were found in ATP content in livers from BD-induced rats and control rats, implicating that the phase of BD up to 6 h does not reduce general liver viability. However, it has also been described that the ATP content after 3 h of reoxygenation after graft harvesting was significantly increased in liver biopsies from brain-dead rats, may be because of a stress response, caused by increased catecholamine levels induced by BD and exaggerated reoxygenation times of liver tissues, exacerbating the I/R injury [39].

Hemodynamic instability, hormonal alterations, blood coagulation factor consumption, lung tissue changes, hypothermia, and electrolyte disturbance generated by BD invariably influence hepatic functions. Increased AST and ALT concentrations indicated liver dysfunction in brain-dead pigs and hepatocyte edema, hepatic sinusoid compression, and other microscopic observations in such animals demonstrated damage to liver cells [40, 41]. Also, BD causes time-dependent general dysfunction in rats, as indicated by elevated LDH and creatinine, AST and α-GST levels [42]. The disruption of liver function seems to be due to circulatory collapse and hemodynamic instability [41]. Morphological changes in the liver following BD are even less well defined. Recent experimental findings show that hepatocytes in livers from brain-dead donors show an altered cell membrane permeability and integrity [17, 43]. It has also been reported increased caspase-3 activity indicating that apoptosis occurs in liver tissue of brain-dead donors [3].
A systemic inflammatory response is present in most patients at the diagnosis of BD [44]. The intense cellular and molecular activation that quickly follows the acute onset of BD involves acute transcriptional upregulation of inflammatory cytokines, both on a systemic and intra-organ level [16]. In liver, aside from microcirculatory changes, early effects of I/R injury and BD are mediated via Kupffer cells. When I/R injury occurs, Kupffer cells and neutrophils are activated, together with an increased expression of adhesion molecules and infiltration of monocytes and lymphocytes [9, 45]. Cell death by necrosis and apoptosis is initiated. In addition to mitochondrial damage, synthesis of oxygen-free radicals is boosted and accompanied by a decreasing activity of antioxidant enzymes. All of this results in immediate organ damage, contributing to hepatic failure after transplantation. In BD models many of these events have been also documented, exacerbating the hepatic damage that already occurs by I/R. In this sense, expression of adhesion molecules in endothelial and epithelial cells, initiating the release of proinflammatory cytokines and the infiltration of immune competent cells is induced by BD [16, 46–50]. VCAM-1 expression was found after 6 hours of BD in both hypotensive and normotensive donors. The pattern of VCAM-1 expression was similar to that described by others during periods of inflammation or rejection of the liver. Also, the ICAM-1 expression observed in all brain-dead rats was similar to expression patterns during episodes of inflammation [42, 51–55]. Leukocyte recruitment to the underlying parenchyma was facilitated, as expected, with upregulated VCAM-1 and ICAM-1 expression, with a significant increase in infiltrating leukocytes in the liver tissue of brain-dead rats. Also, naive as well as activated macrophages (i.e., ED1- and ED2-positive cells) were significantly increased in brain-dead donors versus controls [42]. The detrimental changes observed during experimental BD were confirmed in more limited clinical studies. Inflammatory changes were found in cadaveric donor livers that showed increased proportion of CD3+ lymphocytes, CD68+ monocytes, and macrophages relative to livers from living donors [33, 56, 57]. Clinical events after BD included more frequent acute rejection episodes and increased lymphocytic infiltrates [33, 56]. In human liver transplants, donor BD triggered upregulation of inflammatory cytokines IL-6, IL-10, TNF-α, TGFβ, IFN-γ, and MIP-1α. Those findings correlated with more pronounced cellular infiltrates, a higher incidence of primary graft dysfunction, and frequent acute rejection episodes [16, 58]. Findings in small animal models of BD parallel clinical observations [16], for instance upregulation of IL-10 and iNOS mRNA in liver has been demonstrated after 6 h of BD [39]. On the contrary, another study has not found iNOS induction in brain-dead animals. This contradictory result may be explained by the fact that the phase of BD in this later study was maintained for only 2–3 h [39].

3. Experimental models used to study the effect of brain death on liver graft quality

Future research in experimental models of LT using BD donors is required to understand the pathophysiology of BD and elucidate the consequences of BD on graft function and survival [6]. The knowledge of the underlying mechanisms on the detrimental effects of BD is a critical question to promote better preservation of the organ and to improve outcome after LT [1].
A wide variety of BD models have been described in the literature, and in most of them an increase in intracranial pressure is generated by an expanding intracranial balloon, finally leading to BD [26]. This section will discuss about experimental animal models used to evaluate the effects of BD on optimal and steatotic liver grafts.

3.1. Experimental BD in small animals

Irreversible pontine ischemia is the essential hallmark of experimental BD. As hemodynamic instabilities related to cardiovascular collapse may bias experimental results, invasive monitoring is important for accurate determination of volume and maintenance of physiological blood pressure. The initial spike in blood pressure during brain stem herniation, apnea, and transient spontaneous reflexes with subsequent absence of spinal reflexes are characteristic criteria of the central catastrophe in experimental models of BD [16, 24, 28, 29, 31, 59]. To produce BD in small laboratory animals, a Fogarty balloon catheter (2–4 French) is inserted through a parietal bore hole into the subdural space and inflated. Reported inflation volumes for induction of the condition range from 200 to 500 μL in rats and 80–103 μL in mice. The inflated balloon catheter is left in place during the entire period of observation to avoid intracerebral hemorrhage and hemodynamic collapse. Computerized axial tomography or magnetic resonance imaging in brain-dead animals can document that the catheter inflation causes the hindbrain to herniate through the foramen magnum [16, 21, 28, 29, 60, 61]. Two types have been described: the so-called ‘sudden onset’ BD model and the ‘gradual onset’ model. In the gradual onset model described by [26], induction of BD was started by gradually increasing the intracranial pressure by inflating the balloon with 16 μL saline per minute. During balloon inflation, a hypotensive period occurred followed by a short peak and a subsequent drop in blood pressure. When the blood pressure returned to its basal level during an increasing peak, inflation of the balloon was stopped and anesthesia was withdrawn. The state of BD was confirmed 30 min after the onset of BD, by the absence of corneal reflexes and a positive apnea test, and such condition was maintained during 1 h or for 4 h [26]. In contrast to the sudden onset model, the gradual onset model simulates cerebral hemorrhage by slowly increasing the intracranial pressure, resulting in less hemodynamic instability and maintenance of normotension during BD for several hours. So far, only a few studies in abdominal organs have described BD induction using a gradual expansion of an intracranial balloon [24, 26].

Authors using a sudden onset model of BD [60] have demonstrated upregulations of proinflammatory markers in different organs, including the liver. BD was produced by rapid balloon inflation of a Fogarty arterial embolectomy catheter introduced into the subdural space through an occipital burr hole; this maneuver suddenly increases intracranial pressure and causes herniation of the brain stem within 20 min in all animals. The rats were tracheostomized and mechanically respirated for periods up to 6 h. [60]. Authors conclude that the experimental system described provides a potentially clinically relevant model in which to study the systemic effects of BD in detail and suggests means to prevent changes in peripheral organs [60]. Thus, it has been postulated that sudden onset BD reflects the situation of the hemodynamically instable donors [23]. In a sudden onset’ BD model described by [23], through a
frontolateral trepanation a balloon catheter was introduced in the subdural space with the tip pointing caudally. Inflating the balloon for 1 min increased the intracranial pressure thereby inducing rapid progressive brain injury leading to BD, which was based on the sharp rise and subsequent drop of blood pressure and heart rate. The state of BD was confirmed 30 min after induction by the absence of corneal reflexes and an apnea test and the effect of BD was studied at 1 h and 6 h after BD induction [23]. Hemodynamic changes in this model, mimicking acute isolated cerebral trauma, included first a sudden increase and subsequently a gradual drop of both heart rate and mean arterial pressure [23]. This BD model appears to be a reliable tool to mimic the type of fast occurring brain injury due to isolated cerebral trauma in man. In this BD model that reflects the situation of the hemodynamically instable donor, expression of immediate early genes was found in tissues of liver and kidney coinciding with progressive dysfunction of these organs and suggest a progressive detrimental effect of BD on donor organ quality, especially in livers from hemodynamically instable donors [23].

In these experimental models described above, only the effect of induction of BD on liver tissue was evaluated. Unfortunately, the other conditions (ischemia and reperfusion) that are present in the clinical practice of transplantation were not included. It is important to evaluate such conditions, since they negatively affect liver grafts already damaged by BD when these grafts, especially steatotic ones, are obtained from cadaveric donors.

Given the prevalence of hepatic steatosis in the population, this represents a large potential pool of donors. However, the clinical problem is still unresolved as steatotic livers are more susceptible to I/R injury and, when used, have poorer outcome than non-steatotic livers [6]. To date, only one experimental study has evaluated the effect of BD on optimal and steatotic liver grafts, which were subsequently subjected to cold ischemia and transplantation. In such research [1], authors have tested the influence of hepatic steatosis and BD separately and in combination in an experimental model of LT [1]. A balloon catheter was introduced through the drill hole in the extradural space with the tip pointing caudally. The intracranial pressure was increased by inflating the balloon for 1 min. The increase in intracranial pressure induced rapid brain injury, leading to immediate BD, simulating a condition comparable to acute isolated cerebral trauma in humans. The state of BD was confirmed by the absence of corneal reflexes and an apnea test and liver grafts were extracted from donors at 6 h after the induction of BD [1]. Steatotic and non-steatotic liver grafts were preserved during 6 h and then were transplanted and submitted to reperfusion for 4 h. Authors report for the first time that the injurious effects of BD in LT are exacerbated in the presence of hepatic steatosis and occur before liver grafts are retrieved from donors [1]. In addition, the mechanisms responsible for the detrimental effects of BD were different depending of the type of the liver, which would interfere with protective pharmacological or surgical strategies applied in liver grafts, avoiding its potential benefits [6]. In such a study, BD-induced dysfunction in the cholinergic anti-inflammatory pathway and prevented the benefits induced by ischemic preconditioning, a surgical strategy that shows benefits when it is applied in non-BD clinical situations. In fact, the study indicated that the combination of acetylcholine and ischemic preconditioning could be considered as a feasible and easy-to-perform intervention to reduce the adverse effects of BD and improve the quality of liver grafts. So authors propose that the time frame between the declaration of BD and organ retrieval provides an important window for cytoprotective intervention, which may counteract the detrimental effects of BD [1].
Table 1 summarizes the experimental models used in small animals to study the effect of BD on liver graft quality.

### 3.2. Experimental BD in large animals

In the baboon, BD is produced by creating intracranial hypertension. Under full inhalation anesthesia, a Foley catheter was introduced into the subdural space through a frontal burr hole in the skull and filled with 20–30 mL of saline, then BD occurred within 20 min [62, 63]. BD has been also induced in pigs by ligation of both brachiocephalic arteries, from which arise the carotid and vertebral arteries. Both experimental models of BD led to a series of major pathophysiological changes that may be collectively referred to as the autonomic storm. Though there was a brief initial period of excessive parasympathetic activity, evidenced by a marked bradycardia, most of the effects of this autonomic activity were brought about by the sympathetic nervous system [63].

Porcine BD models have been widely used in transplant surgery studies. A research group [64] performed a study with a lengthening of the induction phase to 60 min with gradual epidural balloon inflation up to 15 mL. Furthermore, they suggested that prolonged BD process might give the necessary preconditions to trigger a systemic inflammatory response that might contribute to organ dysfunction. Compared to the clinical situation, other authors [27] considered that 60 min was still a relatively short time. They therefore wanted to prolong the BD induction phase up to 200 min by stepwise increase of intracranial volume, followed by a 30-min observation period. Analysis of the monitoring results showed a classic intracranial pressure-volume relationship. Furthermore, intracranial compliance decreased gradually when intracranial pressure increased, which reflects a decreasing ability to compensate for added intracranial volume [27]. The described so-called Cushing response with arterial blood pressure increase, bradycardia, and respiratory irregularities was also demonstrated [27]. Authors believe that their model has the advantage of applying gradual progressive changes in intracranial pressure, cerebral perfusion pressure, and brain tissue oxygenation, leading to cellular injury in parenchymatous organs. Therefore, the model provides the possibility

| Small animals |
|----------------|
| Article       | Type of BD       | Time of sustained BD | Support during BD          | Cold ischemia   |
| Ref. [1]      | Sudden           | 6 h                 | Ventilation                | 6 h             |
| Ref. [23]     | Sudden           | 1 or 6 h            | Ventilation                | Not realized    |
| Ref. [26]     | Gradual          | 1 or 4 h            | Ventilation                | Not realized    |
| Ref. [31]     | Gradual and sudden | 6 h               | Ventilation                | Not realized    |
| Ref. [42]     | Sudden           | 1 or 6 h            | Ventilation                | Not realized    |
| Ref. [60]     | Sudden           | 6 h                 | Ventilation                | Not realized    |

Table 1. Experimental models in small animals used to study the effect of BD on liver graft quality.
of studying the effects of BD processes on organ quality and function as well as outcomes after transplantation. Furthermore, the model also may be used for explorative studies of brain injury mechanisms and for evaluating new neuromonitoring devices [27]. In another experimental model [65], BD has been induced by surgically placing an epidural balloon and gradually increasing the inflation to increase intracranial pressure to 15, 25, 35 and 45 mm Hg, maintaining each pressure level for 30 min, in piglets [65]. However, opposite results in comparison with the experimental model using a BD induction phase of 200 min [27] were observed, this means a decrease of the arterial blood pressure and tachycardia. The reason for this difference is unclear, but it is well-known from the clinical situation that a Cushing response is not always observed in patients with BD developing [27].

More recently, [66] have established a clinically relevant, reproducible, large animal model of BD in pigs, based on a controlled cerebral hemorrhage. To induce intracranial hemorrhage, a needle was inserted through a burr hole over the left hemisphere, and stereotaxically placed in the internal capsule at the level of the lateral ventricle. Blood was withdrawn from the central arterial catheter in a 10 ml unheparinized syringe and then was injected in the brain with a rate of 40 ml/h. The infusion of blood continued for 60 min to maintain an intracranial pressure sufficient to ensure BD. Pigs in the control group underwent surgical preparations similar to the BD group, including burr holes and tissue glue but without injection of blood. Computerized tomographic angiography was performed 120–180 min after BD [66]. Irreversible damage to the brain stem was validated by a negative atropine test, disappearance of corneal and pupillary light reflexes, and a negative cerebral perfusion pressure sustained for more than 60 min. The disappearance of intracranial pulse pressure waves supports the diagnosis, as this is a clinical parameter associated with BD [66].

Further investigations will be required to investigate whether all of these experimental models of BD above mentioned are adequate to study the effect of BD on a graft that also presents I/R injury and which one best simulates the conditions present in clinical practice.

Another experimental model of BD in large animals has been reported [67], which has been previously established and neuropathologically validated [19, 67]. In this model the effect of BD on liver grafts also undergoing cold preservation and transplantation was evaluated. BD was induced suddenly by the injection of 8–17 ml of normal saline solution into the catheter balloon over a period of 5–10 min using dogs as experimental animals. This produced a sudden rise in arterial blood pressure (systolic and diastolic) and heart rate, reflecting an explosive intracranial pressure increase and defined as the “Cushing reflex” [67]. Once the diagnosis of BD was established, the dog was monitored for 16 h before the liver was removed [67]. Livers were stored floating in 1000 ml of the UW preservation solution at 4°C for 24 h [67] and at the end of the preservation time, the livers were transplanted orthotopically using a modified technique with a sutured upper caval anastomosis and cuffed anastomoses for the infrahepatic vena cava and the portal vein [67]. Although not all the clinical scenario of BD could be replicated, this experimental model simulates the sudden rise in intracranial pressure caused by acute cerebrovascular lesions or traumatic brain injury [67]. As reflected by the hepatic enzyme release during the course of BD, no evidence of deterioration of function in livers retrieved from BD donors was found. These findings are different from rodent models. In addition, it should be borne in mind that in clinical practice it is well documented that BD negatively
affects the function of liver grafts that are procured from a cadaveric donor and subsequently transplanted [68]. Therefore, to achieve a successful design of therapeutic strategies to protect liver grafts against harmful effects of BD, it is important to take into account only experimental models in which the negative effects of BD on the liver tissue have been demonstrated.

Table 2 summarizes the experimental models in large animals used to study the effect of BD on liver graft quality.

| Article     | Type of BD | Time of sustained BD | Support during BD | Cold ischemia |
|-------------|------------|---------------------|------------------|---------------|
| Ref. [27]   | Gradual    | 1 h                 | Ventilation      | Not realized  |
| Ref. [64]   | Gradual    | 6 h                 | Ventilation      | Not realized  |
| Ref. [65]   | Gradual    | 30 or 60 min        | Not described    | Not realized  |
| Ref. [66]   | Gradual    | 8 h                 | Not described    | Not realized  |
| Ref. [67]   | Sudden     | 6, 12 and 16 h      | Inotropic support| 27 h          |

Table 2. Experimental models in large animals used to study the effect of BD on liver graft quality.

4. Conclusions

Most organs for transplantation originate from BD donors. Although the detrimental consequences of BD have been clinically described, the underlying mechanisms and their relevance in transplantation remain poorly understood. Indeed, few studies have evaluated the effect of BD on transplantation, and, as stated along this chapter, most of the experimental studies focused in the pathophysiological changes occurring during BD without transplantation. Moreover, these studies have been performed in the different sudden and gradual BD models, so dissimilar results on pathophysiological changes and treatments have been described.

Comparison of the results of animal studies and their extrapolation to human beings is feasible, but with limitations such as differences in BD and ischemia tolerance, anatomy of the liver of various species, surgical conditions used in clinical practice and those used in the experimental models. Importantly, studies performed in small animals are of limited applicability to human beings due to their different size and anatomy of the liver and their faster metabolism. Large animals exhibit greater similarity in their anatomy and physiology to humans; however, their use is restricted by serious logistical and financial difficulties, ethical concerns and limited availability of immunological tools for use in large animal species. In addition, in some cases, experimental models of BD in large animals did not mimic the clinical conditions in liver transplantation from BD. Despite the limitations of the experimental animal models, these are the best options to study hepatic I/R, especially considering that the progress of human studies is slow, the majority of human tissues are not routinely accessible for research, and there is very limited opportunity for interventional studies. The clinical application of strategies
designed at benchside will depend on the use of experimental models of BD that resemble as much as possible the clinical conditions that happens in BD and LT [69]. Table 3 summarizes the reproducible clinical effects of BD that happens in the different experimental models.

We recognize the complication, but future research in similar experimental models of transplantation using BD donors is required to understand the pathophysiology of BD and elucidate the consequences of BD, especially in sub-optimal liver grafts. Thus, multidisciplinary research groups should devote additional efforts to better understand the pathophysiology of BD to ultimately develop effectual therapeutic strategies aimed at improving graft viability, and at significantly increasing the organ donor pool.

### Acknowledgements

This research was supported by the Ministerio de Economía y Competitividad (project grant SAF-2015-64857-R) Madrid, Spain; the European Union (FondosFeder, “unamana de hacer Europa”); by CERCA Program/Generalitat de Catalunya; by the Secretariad’Universitats i Recerca (Grant 2017SGR-551) Barcelona, Spain; and the Consejo Nacional de Ciencia y Tecnología (CONACYT) (Fondo Sectorial de Investigación para la Educación, Grant 257743), México. ME Cornide-Petronio has a Sara Borrell contract from the Instituto de Salud Carlos III (Grant CD15/00129), Madrid, Spain. MB Jiménez-Castro has a contract from the Programa de Promoción del Talento y su empleabilidad from the Ministerio de Economía y Competitividad (Grant EMP-TU-2015-4167), Madrid, Spain.

### Conflict of interest

The authors declare that they have no conflict of interest.
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