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MINIREVIEWS

Potential role of the gut microbiota in synthetic torpor and therapeutic hypothermia

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Abstract

Therapeutic hypothermia is today used in several clinical settings, among them the gut related diseases that are influenced by ischemia/reperfusion injury. This perspective paved the way to the study of hibernation physiology, in natural hibernators, highlighting an unexpected importance of the gut microbial ecosystem in hibernation and torpor. In natural hibernators, intestinal microbes adaptively reorganize their structural configuration during torpor, and maintain a mutualistic configuration regardless of long periods of fasting and cold temperatures. This allows the gut microbiome to provide the host with metabolites, which are essential to keep the host immunological and metabolic homeostasis during hibernation. The emerging role of the gut microbiota in the hibernation process suggests the importance of maintaining a mutualistic gut microbiota configuration in the application of therapeutic hypothermia as well as in the development of new strategy such as the use of synthetic torpor in humans. The possible utilization of tailored probiotics to mold the gut ecosystem during therapeutic hypothermia can also be taken into consideration as new therapeutic strategy.

Key words: Therapeutic hypothermia; Gut microbiota; Hibernation; Dysbiosis; Synthetic torpor

Core tip: Therapeutic hypothermia is currently limited by several factors, including the patient compensatory response. Recently, the possibility to induce synthetic torpor in non-hibernators has opened new scenarios, but a deep understanding of torpor and hibernation physiology is required. One particular aspect to consider

is the gut microbiota (GM). In hibernators, the GM undergoes seasonal shifts, as a physiological response to the body temperature drop, and is considered playing a central role in regulating physiology, specifically influencing the immune system. Since the GM changes induced by synthetic torpor have never been described in non-hibernators, possible risks and reasonable interventions are suggested.

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THERAPEUTIC HYPOTHERMIA AND SYNTHETIC TORPOR

Generality

Therapeutic hypothermia (TH) is the procedure to reduce body temperature in patients for therapeutic purposes[1,2]. The rationale behind the use of TH is to reduce the energy requirement for cells in order to counteract a condition of severe lack of energy supply. In conditions such as cardiac arrest[3], or stroke[4,5], TH can be beneficial, reducing cellular metabolism in neurons, therefore reducing the extent of the damage. Currently, TH is induced by physical means, and cooling is achieved by direct infusion of cold fluids and by cooling of the skin[6]. The application of TH in clinical practice is limited by two main factors: first, the degree of cooling has to be modest, usually not below 34 ℃, since possible cardiac side effects can arise from lower temperatures[5]; second, the brain tends to defend the body temperature against cooling, for instance by activating shivering[6]. Such defence may lead to an increase in energy expenditure, that can even counteract the searched positive effects of TH. To improve the use of TH, the development of a procedure to induce a state of undefended, deep hypothermia would be very useful. A promising path would be to mimic the natural state of torpor, inducing a state of undefended hypometabolism that was recently referred to as synthetic torpor[7].

Torpor, hibernation and aestivation

In nature, a special case of undefended hypothermia can be observed in mammals during torpor. Torpor is a bout of metabolic suppression[8] that leads to an undefended decrease in body temperature according to the thermal gradient with the environment. Such metabolic suppression can last from a few hours to many months. Short-lasting bouts of metabolic suppression are referred as daily torpor, whereas longer bouts, usually interrupted by brief interbout arousal, are referred as hibernation, when occurring at low ambient temperature, or aestivation, when occurring at higher ambient temperature[9,10].

During hibernation, many organs present specific adaptations to this condition. The brain synapses are retracted[11,12] and neurons express an hyperphosphorylated Tau protein[13]. Heart rate is strongly reduced and rhythm is not regulated[14]. Lung tissue is remodeled[15]. Thrombocytopenia is also observed and lymphocytes are segregated into lymph nodes[16]. Muscles do not lose mass and strength after long periods of immobilization[17]. Bones of hibernators also maintain strength and structural properties[18]. During hibernation, cell proliferation is stopped, a process also seen in cancer cells[19]. Moreover, hibernators have an increased radioprotection from X-ray and gamma radiation[20]. The anatomy of the gastrointestinal tract (GIT) is also affected during hibernation, with an increased leakage of the intestinal barrier and some lymphocytic infiltration in the mucosa; cell proliferation in the intestinal crypts is also arrested[21].

Synthetic torpor in non-hibernators and clinical applications

Developing a procedure to induce synthetic torpor in non-hibernating animals, including humans, would be advantageous in many clinical settings[7]. Currently, only two procedures were shown to be effective to induce synthetic torpor in non-hibernators (rats): Cerri and coworkers first showed that the activation of the $GABA_A$ receptors on neurons within the Raphe Pallidus (RPa), a region on the ventral surface of the brainstem, was sufficient to induce a state of deep hypothermia accompanied by bradycardia, reduced electroencephalogram (EEG) activity, and no significant changes in arterial pressure[22]; Tupone and coworkers showed that also the intracerebroventricular injection of the agonist for the adenosine A1 receptors, N6-cyclohexyladenosine, induces a drastic drop in core temperature, heart rate and EEG activity[23]. In both the previous researches, the procedure was shown to be safe and reversible, hypothermia was maintained for 6 h, and no side effects were reported[22,23]. Both the procedures can be considered as a proof of concept that a synthetic torpor could be induced even in humans, but, even if more work is required to achieve such goal, several evidences suggest that it may be possible[7,24]. Evolutionarily, torpor is in fact a trait that was probably present in the proto-mammal, since most orders of mammals[25], including primates[26], present it. The trait was subsequently lost in many mammals, remaining present in all the species relying on energy saving to survive. It can be argued that the set of genes necessary to survive a deep hypothermic state is shared among mammals. Also, an interesting case of spontaneous torpor was reported in humans[27], strengthening the idea that synthetic torpor could be induced in humans.

The cytoprotective effects of torpor could be very useful in several clinical conditions, among which is transplantation medicine: the possibility of reducing donor organ metabolism could lead to a better preservation of the organ itself, compared to the cold storage[28-30]. For patients with short bowel syndrome, or untreatable bowel diseases, intestinal transplantation is a common approach[28]. However, problems related to ischemia/reperfusion injury, and prolonged cold storage may affect the success of the transplant[28]. One typical consequence of extended cold preservation consists in the alteration of cellular structure, which leads to the disruption of mucosal barrier, then resulting in the disruption of the typical absorptive, and protective roles of the intestine[28]. Such consequences do not appear after torpor, because of the protecting factors activated, making torpor a valid alternative approach in cases of organ preservation.

The pathogenesis of many gut related diseases, such as acute mesenteric arterial occlusion, neonatal necrotizing enterocolitis, midgut volvulus, and septic shock, is influenced by ischemia-reperfusion injury[31]. The reperfusion is generally associated with systemic inflammation and bowel injuries, and leads to multiple organ impairment, showing high mortality rate[32]. One possible approach suggested to contain reperfusion derived damages, consists in the application of whole body moderate hypothermia[33-35]. Specifically, this technique was proven to not only limit injuries on the reperfused gut, but it avoids multiple organ dysfunction syndrome, and it was proposed as a rescue therapy for intestinal ischemia/reperfusion injury[34]. It is possible to speculate that the whole body hypothermia can be more beneficial over selective cooling, and it enables a systemic control over the inflammatory state, leading to a reduction of metabolic needs.

In order to safely use synthetic torpor in humans, both maximizing the benefits and containing possible side effects, it is necessary to understand hibernation physiology. Moreover, in cases of gut injuries, it is important to have a clear understanding of the gut role during hibernation.

Importance of the gut in hibernation physiology

During hibernation, the intestine, especially the proximal tract, undergoes a severe atrophy, without changing the overall structure[36]. It means that the transport of nutrients and electrolytes is depressed, but the fact that the overall structure is well conserved allows the intestine to perform normally during the interbout arousals[37]. One hypothesis to explain the arousals is that they allow solute transport during torpor, so that the animal is able to use microbial derived short chain fatty acids (SCFAs), vitamins and other substances in the lumen[38]. During the fasting period typical of hibernation, the microbes living in the gut have access to only host derived compounds, such as mucus (glycans and proteins), and epithelial cells[39]. These products cannot be degraded efficiently during torpor, but interbout arousals boost enzyme activity, and the consequent production of SCFAs[40,41]. The SCFAs during hibernation are valuable for the host: their metabolism leads to ATP synthesis, or they are converted in ketone bodies as energy alternatives to glucose, or they can boost gluconeogenesis pathway[37]. Furthermore, SCFAs offer to the intestinal epithelial cells 70% of the necessary energy for cell proliferation. Thus low levels of SCFAs during torpor may be central for the atrophy recorded in the small intestine mucosa[37].

The gut immune system shows alterations during hibernation, indeed gut epithelium becomes leakier during the long torpor phase, leading to the uncontrolled passage of many molecules and even of bacteria[39]. The increased permeability risks to determine a hyper activation of the immune system in hibernating mammals, but interestingly, although a higher number of immune cells is found, the mucosa does not appear damaged, and there are no signs of pathology[39]. One hypothesis explaining these discoveries, proposes that the immune system is stimulated to re-organize during hibernation, thanks to changes in the gut microbiota (GM) composition. Thus, it seems that the immune system can provide a higher tolerance, avoiding overactivation of the immune response in this increased permeability condition[42].

GUT MICROBIOTA IN THERAPEUTIC HYPOTHERMIA

The gut microbiota: structure and function

The total amount of symbiotic bacterial cells living in the human body varies between 10 and 100 trillion in each person[43], but the highest concentrations occur in the GIT, generating one of the densest and most biodiverse ecosystems known so far: the human GM[43]. The GM constitutes a biomass of 1.5 kg[44], and is particularly concentrated in the colon tract, with a density of 10^{12} CFU/g of luminal content. Regarding its phylum-level taxonomic layout, the GM shows a general common population: Firmicutes (mean relative abundance, 65%), Bacteroidetes (25%), Actinobacteria (5%), Proteobacteria (< 8%), Verrucomicrobia, and Fusobacteria (1%)[45]. The biodiversity of the GM is more appreciable at species level: more than one thousand different species have been detected in the GM of the human population[46]. The GM varies among the population, indeed a healthy subject hosts a specific subset of hundreds of species[46], resulting in a high inter-individual variability, which may depend on several factors, as genetics, age, environment, diet, and it is not stable during adulthood, but always prone to changes[47]. The presence of these huge amounts of bacteria in the human gut determines a relevant genomic contribution (generally referred to as microbiome), which overcomes the human genome of at least

100 fold, and it provides functions that human beings have not evolved on their own, including metabolic, physiological and immunological support[44].

One of the most evident advantages for the host, is the ability of the GM to encode for enzymes involved in carbohydrate metabolism. In this way, the host is able to extract and absorb energy from polysaccharides, such as dietary fiber, otherwise indigestible[48]. Also, supporting the human diet, the microbiome provides vitamins, cofactors and secondary metabolites, which are instead poorly encoded by the human genome[49,50]. Indeed, according to metagenomics analysis, the gut microbiome is enriched for Kyoto Encyclopedia of Genes and Genomes categories involved in metabolism, especially in carbohydrate metabolism, energy metabolism, generation of SCFAs, amino acid metabolism, biosynthesis of secondary metabolites, and metabolism of cofactors and vitamins[48,51]. Moreover, the GM has a protective action against colonization by enteric pathogens, solving this function in different ways: first of all, the presence of dense colonies on the GIT surface represents a direct inhibition of pathogen growth; indeed, the GM not only determines the depletion of nutrients, making the GIT a competitive environment, but it also produces substances itself, against pathogens, including bacteriocins, and proteinaceous toxins[52,53].

The GM is an extremely plastic and adaptive ecosystem, influenced by both host genome and environmental factors[53-55]. Specifically, the GM is mostly affected by changes in the environment, together with host habits, showing a profound adaptation to new contexts[56]. A change in dietary habits is what influences the GM composition the most. The GM structure is altered after only 24 h of dietary shift, and the change is driven also by the kind of nutrients assumed: these results together point out the extreme plasticity of the GM in responding to dietary changes[57,58]. Interestingly, both short-term and long-term dietary changes were confirmed in a recent study and, in addition, a taxon-nutrient correlation was highlighted[59]: this finding associated specific bacterial taxa with the intake of a specific class of nutrients, showing a bacterial composition shifting in a reciprocal and inverse pattern, respect to the intake of animal, or plant derived products. The ability of the GM to quickly adapt in response to new dietary habits, suggests that it offers the host the capability not to suffer from a dietary alteration, instead taking the advantages of it, by improving host metabolism, and favoring extraction of specific dietary substrates.

The presence of this incredible amount of bacteria in the GIT also represents a potential risk for the individual health, suggesting a deep involvement of the immune system[60,61]. The immune system solves a crucial role in maintaining the homeostatic relation between host and GM, limiting bacterial invasion, and protecting against pathogens, otherwise leading to severe illnesses, such as inflammatory bowel disease[62]. The immune system regulates intestinal homeostasis on multiple levels: first of all, it limits the bacteria-epithelial surface contact; it identifies and eliminates microbes infiltrated in mucosal tissues; it minimizes immune system exposition to residential bacteria, avoiding the hyper activation of the immune system that may lead to auto-immune disorders[62]. Moreover, it is important to consider that the presence of the GM determines an adaptive response of the immune system. Dendritic cells underneath the epithelial layer extend their projections between epithelial cells, to sample the bacterial composition of the intestinal lumen. Then, they activate lymphocytes B in Peyer's patches to differentiate into IgA-producing cells, which in turn will be released in the lumen[63]. Such activation is specifically directed to intestinal microbes, to limit their interaction with the epithelium[64], and to prevent their penetration under the epithelial layer[65]. This intense GM-host immunological dialogue plays a strategic role for the regulation and the development of the immune system, indeed studies on germ free mice have shown the microbiota to regulate and shape the Gut-Associated Lymphoid Tissue (GALT)[62]. Furthermore, the presence of specific microbial groups, such as fiber fermenting SCFA producers, from the *Clostridium* clusters Ⅳ and XIVa, and Bacteroidetes, induces active proliferation of Treg and specific subsets of lymphocytes involved in the synthesis of anti-inflammatory cytokines, supporting the immunological homeostasis through the body[66-68]. Indeed, the absence of this kind of cytokines is known to lead to chronic inflammation of the GIT, characterized by neutrophil and lymphocyte infiltration in the mucosa, increasing in cellular proliferation, with a corresponding increase in mucus secretion[69,70]. This active role of the GM in shaping the immune system has been analyzed further with comparative studies, where germ free mice were colonized with specific bacterial taxa, to observe the immune system remodeling[63,71,72].

Adaptive GM changes in torpor and hibernation

As mentioned earlier, the main adaptive changes in the GM structure depend on the host dietary shifts, and hibernation is an extreme example of this shift. Thus, not only the animal overeats during the summer to store fat and energies, but it shifts to a total starvation lasting long periods during winter, and both the two behaviors affect the GM composition and function (Figure 1). Evidences on hibernating squirrels show that the hibernation cycle strongly affects the luminal microbiota in the animal cecum: during the starvation period, the microbial biodiversity decreased significantly, and specifically a decrease in Firmicutes, and an increase in Bacteroidetes and Verrucomicrobia were observed[40,42]. The change detected in the microbial community is consistent with the necessity for the GM, during hibernation, to survive only on host substrates, such as mucus (glycans and proteins), and epithelial

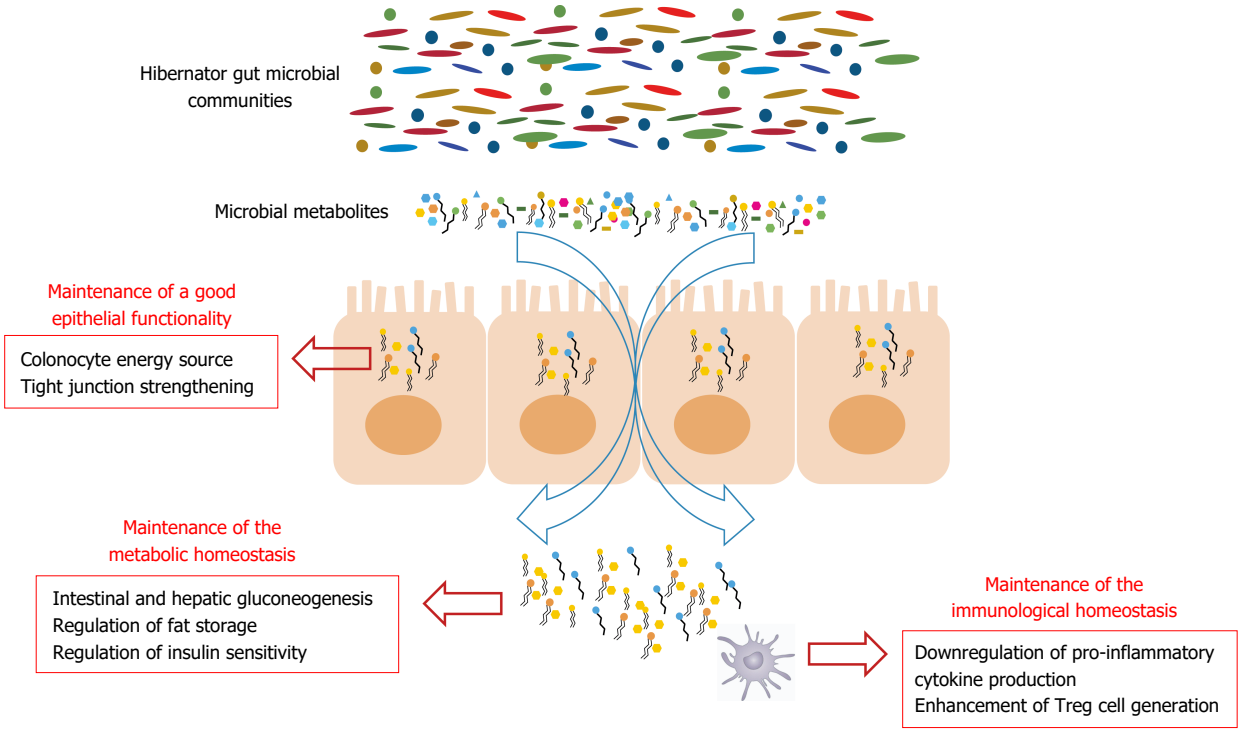

Figure 1 Role of the gut microbiota in the hibernation physiology. In natural hibernators, the gut microbial ecosystem is capable of an adaptive response along the hibernating cycle. Variations in the gut microbiome structure during torpor are functional for several physiological aspects involved in hibernation, such as the maintenance of the good functionality of the intestinal epithelium and the regulation of the metabolic and immunological homeostasis. During interbout arousals, metabolites from a cold and fasting-adapted gut microbial ecosystem reach the enterocytes, the underlying immune cells, as well as the circulation. In each district, the microbial metabolites exert essential functions for the maintenance of the host homeostasis along the hibernating period. In particular, the microbiome derived short chain fatty acids are of strategic importance, being at the same time, a valuable energy source for enterocyte metabolism, a regulator of gluconeogenesis, fat storage, and insulin sensitivity, and, finally, a potent immunomodulator, favoring an anti-inflammatory immune system layout.

cells, as mentioned earlier[73]. These data suggest that the limited amount of substrates drives some microbial species to grow more over others, less able to use host substrates.

Recently, a study in mice focused on the alteration of the GM after cold exposure[74]. Although the mouse is a mammal that can enter into torpor, in case of food scarcity and low temperatures[75], the authors prevent the animal from doing it. First, they observed a shift in the GM of animals exposed to cold temperatures: such shift was consistent with previous studies on ground squirrels[40,43], in particular they recorded changes in Firmicutes:Bacteroidetes ratio. Also, the authors restricted food access in one group exposed to cold, and the animals, according to their nature, tended to enter torpor, reducing their body temperature. Surprisingly, the same effect on body temperature was recorded in animals treated with broad range antibiotics, disrupting the GM, despite unaltered food intake. Such result underlines that energy harvested through food intake, in cold environments, is necessary for maintaining body temperature, but unexpectedly, the result emphasizes the GM contribution in this process. Then, to investigate the importance of the changes in bacterial composition as an adaptive response to the cold exposure, the GM from mice after 4 wk of cold exposure, was transplanted in germ free mice. The effects of the transplant were surprising, including an increase in insulin sensitivity, increase in brown adipose tissue activity markers, and browning of the white adipose tissue. Moreover, to compare cold transplanted mice to controls, they were both exposed to extreme cold environment, and while controls reduced their body temperature after 4 h, cold transplanted mice were fully resistant to cold temperature, increasing the thermogenesis. Interestingly, the quantity of SCFAs increased after the transplant, compared to germ free mice transplanted with warm GM. Thus, the increased fermentation after transplant, has been proposed to be associated to the microbial shift during cold, increasing energy harvest. Focusing on morphology, the intestinal length in cold-exposed mice, increased of 35% compared to controls, specifically, at the small intestine level, resulting in an incredible plasticity after only 9 d of cold exposure. The same morphological effects were recorded in germ free mice, transplanted with cold GM, meaning that the microbiota alone contributes to this phenotype[74].

The first study analyzing the alteration of the GM of a free range hibernating bear was recently published[76]. The main aim of the study was to understand bear physiology since, gaining weight during the summer, it develops obesity, which is alternated to weight loss due to the fasting period during hibernation season.

The authors analyzed if the changes in the GM composition may help somehow the bear to not develop insulin resistance, despite the obese phenotype. Fecal microbiota during winter was constituted by less taxa, and it was more homogenous compared to summer microbiota, moreover, according to previous studies, an increase of Bacteroidetes over Firmicutes was recorded. Following these observations, the authors analyzed possible changes in host metabolism, driven by the seasonal differences in the GM. To this aim, germ free mice were colonized with summer and winter bear GM: those colonized with summer GM showed an increase in adiposity, but no alteration in glucose metabolism, on the other hand, winter GM did not induce particular changes in adiposity composition. In humans an increased adiposity leads to insulin resistance, which if not treated, could evolve in type 2 diabetes[77]. Such effect was not recorded neither in the brown bear, during the active phase, nor in germ free mice colonized with summer bear GM. These results suggest the GM might play a central role in maintaining bear metabolic homeostasis, during the transition from the weight gain summer period to the fasting hibernating winter[76].

Watching over the GM in therapeutic hypothermia

It is possible that the GM in natural hibernators has evolved in parallel with its host physiology, in order to adapt to the hypothermic state. Obviously such coevolution is absent in mammals that do not naturally enter torpor, and the drastic temperature drop may determine alterations in the GM, and in the gut physiology in general, totally different from what is observed in hibernators. An inflammatory state is easy to develop in case of a leaking intestine, condition that appears related to gut atrophy typical of hibernation. In natural hibernators, the inflammation is contained thanks to changes in the immune system itself, together with the altered cross-talk with the GM. Such physiological changes must be evaluated in non-hibernating animals, in fact, the lack of the modulation of the immune system may lead to an unfavorable condition such as inflammation. In inflammatory conditions, opportunistic pathogens can easily impair the host-microbiota relationship, leading to a dysbiotic layout with an alteration of all the functions dependent on the GM-host mutualism. This risk should stimulate future studies to take into account GM changes in non-hibernating animals, on which is applied synthetic torpor. Analyzing the changes induced by the induction of a synthetic torpor in the gut of non-hibernating mammals, offers the chance to make synthetic torpor safer, avoiding the development of inflammation and dysbiosis.

A possible way to solve this condition might be the rational manipulation of the GM toward a hibernator-like configuration usage. This can be achieved by mean of dietary-based approaches, the utilization of prebiotics, as well as next generation probiotics. In this scenario, our suggestion for a possible future clinical use of synthetic torpor is to focus on GM alteration. Also, in the probable case of dysbiosis, the application of tailored probiotics during the state of synthetic torpor may help to reduce the inflammation, avoiding the arise of eventual side effects.

CONCLUSION

Taken together, these pioneer studies exploring the role of gut microbes in torpor and hibernation demonstrate that, in hibernating mammals, the GM has a strong influence on host physiology, modulates their metabolism, and contributes to the adaptation to the extreme environmental changes. Such role of the GM highlights that in the use of therapeutic hypothermia as well as in the development of a procedure to induce torpor in humans, the GM composition needs to be monitored, and possibly tuned on the hibernation modality.

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