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Protection against renal ischemia–reperfusion injury through hormesis? Dietary intervention versus cold exposure☆

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ABSTRACT

Aim: Dietary restriction (DR) and fasting (FA) induce robust protection against the detrimental effects of renal ischemia–reperfusion injury (I/RI). Several mechanisms of protection have been proposed, such as hormesis. Hormesis is defined as a life-supporting beneficial effect resulting from the cellular responses to single or multiple rounds of (mild) stress. The cold exposure (CE) model is a stress model similar to DR, and has been shown to have hormetic effects and has proved to increase longevity. CE is considered to be the most robust method to increase metabolism through activation of brown adipocytes. BAT has been considered important in etiology of obesity and its metabolic consequences.
Materials and methods: Since DR, FA, and CE models are proposed to work through hormesis, we investigated physiology of adipose tissue and effect on BAT in these models and compared them to ad libitum (AL) fed mice. We also studied the differential effect of these stress models on immunological changes, and effect of CE on renal I/RI.
Key findings: We show similar physiological changes in adiposity in male C57Bl/6 mice due to DR, FA and CE, but the CE mice were not protected against renal I/RI. The immunophenotypic changes observed in the CE mice were similar to the AL animals, in contrast to FA mice, that showed major immunophenotypic changes in the B and T cell development stages in primary and secondary lymphoid organs.
Significance: Our findings thus demonstrate that DR, FA and CE are hormetic stress models. DR and FA protect against renal I/IR, whereas CE could not.

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1. Introduction

Ischemia–reperfusion injury (I/RI) is inevitable during organ transplantation, and is negatively correlated with clinical outcome and survival. In the light of donor organ shortage, extended criteria donors are increasingly being accepted for transplantation, with an increased risk for I/RI. Therefore, novel methods to prevent or reduce I/RI are of paramount importance. We have previously shown that both 14 days of 30% dietary restriction (DR) as well as 3 days of preoperative fasting (FA) protect against renal and hepatic I/RI in mice [1].

DR is considered a reduction in energy intake by 20–40% without causing malnutrition [2], and is one of the most robust, reproducible, non-invasive, clinically applicable and simple experimental manipulations that is known to extend both median and maximum lifespan in laboratory animals [3] and non-human primates [4]. The effect of DR has also proved to be highly beneficial in reducing many aging related diseases, such as diabetes, autoimmune and respiratory diseases [5] along with improvement in kidney disease.

Over the last several decades, research has been focused intensively on trying to elucidate the mechanism behind the beneficial effects of DR. Several studies have demonstrated that DR helps in improving glucoregulatory function and insulin sensitivity along with decreased fasting glucose and insulin levels [6,7]. Other studies have highlighted the role of reduced T-lymphocyte production and proliferation, and slow thymic involution in the beneficial effects of DR [8,9]. We have recently shown that DR causes major immunophenotypic changes in the bone marrow (BM) and thymus, and arrests their B and T cell development [10].

From another point of view, DR and FA have been postulated to reprogram the immunological profile, and induce hypothalamic–pituitary–adrenal (HPA) axis activity, since plasma corticosterone concentrations are increased [11]. As such, DR can be considered a mild stressor commonly referred to as hormesis, defined as a life-supporting beneficial effect resulting from the cellular responses to

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single or multiple rounds of (mild) stress [12]. There is evidence that an increased ability to cope with damage enhances longevity, probably by retarding the damage from aging processes [13,14]. Thus, induction of hormesis by DR and FA might be one of the underlying mechanisms by which these dietary interventions improve I/RI outcome.

Hormesis is promoted by different types of stress that induce different mechanisms. Another model that has shown to have hormetic effects, and has proved to increase longevity is cold exposure [15]. Cold exposure (CE) is a commonly used method to increase metabolism, since it results in activation of brown adipose tissue (BAT) and gives the white adipose tissue (WAT) more BAT-like characteristics. In mammals, BAT and WAT have opposite effects, but both are considered important in etiology of obesity and its metabolic consequences [16]. While white adipocytes in WAT are mainly involved in energy storage in the form of triglycerides (TGs) that can be utilized during the periods of enhanced energy demand, brown adipocytes in BAT can oxidize TG-derived fatty acids to generate heat. BAT is capable to perform the latter since it contains uncoupling protein 1 (UCP1) that, when active, will uncouple mitochondrial oxidative phosphorylation from ATP synthesis. As a consequence, energy is released as heat [17].

In mice, CE also induces HPA axis activity, since it results in elevated plasma ACTH and corticosterone concentrations [18]. Thus, three different models, namely FA, DR and CE, induce HPA axis activity. However, only two of these preconditioning strategies have thus far been shown to ameliorate I/RI: DR and FA. On the other hand, only CE has been shown to result in activation of BAT and appearance of WAT with BAT-like characteristics. With respect to the immunological profiles, changes herein that might be of relevance for I/RI survival have only been studied in the FA and DR animal model [10].

We hypothesize that the temporary induction of stress in either one of the three models is crucial in the protection against I/RI. To study this, we compared the effects of FA, DR and CE on the HPA axis, and whether this correlates with I/RI survival. In addition, to compare the models in more detail, we investigated whether FA and DR affect BAT and whether CE affects the immunological profile and cytoprotective genes.

2. Materials & methods

2.1. Animal models and experimental setup

Male C57Bl/6 mice of 8 weeks, weighing 20–25 g, were purchased from Harlan, Horst, the Netherlands. Mice were kept at normal laboratory physiological conditions with 3 animals per cage having free access to food (Hope Farms, Woerden, the Netherlands) and water until the start of experimental procedures.

For CE, the mice were acclimatized for 1 week under standard housing conditions before being housed individually, and put in a temperature-controlled climate chamber (Bronson, Nieuwkuijk, the Netherlands) with normal light/dark cycle. Body weights and food intake were measured before and after 24 h-exposure to temperatures of either 23 or 4 °C (n = 8/group).

In the dietary intervention group, the animals were divided into 3 groups, namely, ad libitum (AL), 2 weeks 30% DR and 3 days water-only fasting (FA) (n = 8/group). Dietary interventions were performed by Shushimita et al. as previously described [10]. However, for the AL group, mice were divided into two separate groups. One group had 4 animals/cage (Normal) while the other group had 1 mouse/cage (to mimic the cold- and warm-exposure experimental setup) referred to as normal separated. The experiments were performed upon approval by the Dutch National Experiments on Animals Act, complied with Directive 2010/63/EU of the Council of Europe.

2.2. Tissue sample collection

Mice were terminated through exsanguination by cardiac puncture under isoflurane anesthesia. Plasma and tissues such as brown adipose tissue (BAT) and liver were collected and either immediately frozen and stored at −80 °C or fixed in 4% paraformaldehyde, and after 24 h stored in 70% ethanol, and later embedded in paraffin.

2.3. Serum corticosterone measurement

The serum corticosterone concentration of the experimental animals was measured by ELISA (Enzo Life Sciences, Antwerp, Belgium) according to manufacturer's instructions.

2.4. Histology of BAT

To perform the histological analysis of the BAT, hematoxylin and eosin staining was performed on the paraffinized tissues. The paraffin embedded tissues were cut into 8 μm thick slices that were mounted on glass slides. The paraffinized tissue slides were deparaffinized by putting in xylene solution (2×, 5 min each). The slides were then allowed to rehydrate using 100% alcohol (2×, 5 min each), and were put in 96% alcohol for 5 min, followed by 70% alcohol for 5 min. The slides were then washed in aquadest for 5 min and stained with Mayer's hematoxylin solution for 2 min. After hematoxylin staining, slides were washed in running tap water for 10 min followed by counterstain with eosin solution for 4 min. The slides were then dehydrated in 96% alcohol (2×, 2 min each) followed by 100% alcohol (2×, 2 min each) and then cleared in xylene solution (3×, 2 min each). The slides were then covered with PERTEX ® (Histolab, Gothenburg, Sweden), and a second glass.

2.5. Immunohistochemistry (IHC) for Ucp1

For Ucp1 IHC, the 8 μm thick paraffin slices were mounted on 3-aminopropyltriethoxysilene coated slides. These slides were put at 60 °C for 60 min, after which the tissues were deparaffinized in xylene for 6 min, and rinsed 2× with 100% ethanol. The endogenous peroxidase activity of the adipose tissues was blocked using 3% H_2O_2 in methanol for a period of 20 min. After endogenous peroxidase blocking antigen retrieval was performed using fresh NaOH buffered citric acid, and heating it 3× for 5 min followed by cooling down the slides to room temperature (RT). After antigen retrieval the slides were incubated with goat serum (5%) for 5 min at RT, and were then incubated overnight with primary antibody rabbit polycolonal anti-Ucp1 (Sigma-

Table 1
Primer sequences of the genes.

Gene	Sequence, 5′ to 3′	GenBank acc. no.	Reference
Atgl	F:GAGCCCCGGGGTGGAACAAGAT R: AAAAGGTGGTGGGCAGGAGTAAGG		
B2m	F:ATCCAAATGCTGAAGAACGG R:CAGTCTCAGTGGGGGTGAAT	NM_009735	
Cd36	F: CCTCCAGAATCCAGACAACC R: CACAGGCTTTCCTTCTTTGC		[30]
Fkbp5	F: ATTTGATTGCCGAGATGTG R: TCTTCACCAGGGCTTTGTC		[31]
Gr	F: CCGGGTCCCCAGGTAAAGA R: TGTCCGGTAAAATAAGAGGCTTG		
Leptin	F: CAGGATCAATGACATTCACACA R: GCTGGTGAGGACCTGTTGAT		
Lpl	F: GCTGGTGGGAAATGATGTG R: TGGACGTTGTCTAGGGGGTA		
Mcad	F: GATCGCAATGGGTGCTTTTGATAGAA R: AGCTGATTGGCAATGTCTCCAGCAAA		[32]
Pgc1a	F: CCCTGCCATTGTTAAGACC R: TGCTGCTGTTCCTGTTTTC	NM_008904	[33]
Prdm16	F: GACATTCCAATCCCACCAGA R: CACCTCTGTATCCGTCAGCA		
Rn18s	F: GTAACCCGTTGAACCCCATT R: CCATCCAATCGGTAGTAGCG	NR_003278	
Ucp1	F: GGCCTCTACGACTCAGTCCA R: TAAGCCGGCTGAGATCTTGT	NM_009463	[34]

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