# New therapeutic and diagnostic approaches with which to influence mitochondrial dysfunctions

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# I. INTRODUCTION

*1.1.1. Mitochondria, characteristics and functions*

Mitochondria are membrane-enclosed organelles, the energy-producing centers in almost all eukaryotic cells. The outer membrane fully surrounds the inner membrane, with a small intermembrane space between, and the inner membrane is loaded with protein complexes of the mitochondrial electron transport chain (METC) and adenosine triphosphate (ATP) synthesis. This membrane surrounds the matrix, where the citric acid cycle produces the electrons that travel from one protein complex to the next. The outer membrane has many protein-based pores that allow the passage of ions and molecules as large as a small protein. In contrast, the permeability of the inner membrane is restricted, much like the plasma membrane of a cell.

The METC is of decisive functional importance: it is responsible for all the fundamental physiological processes of the cell, producing chemiosmotic energy through $O_2$ consumption. In brief, electrons are transported through the complexes of the METC and finally accepted by the $O_2$, this process leading to ATP synthesis, the exclusive source of cellular energy.

*1.1.2. The Mitochondrial oxidoreductive stress*

Oxidative stress is generally defined as an imbalance that favors the production of reactive oxygen species (ROS) over antioxidant defenses; this term is applied to *in vivo* situations in which damage is caused by an elevated level of ROS or other free radical generation, either directly or indirectly. Nevertheless, the majority of ROS are products of mitochondrial respiration. If only one electron is utilized for the reduction of $O_2$, a relatively stable intermediate is produced, a superoxide anion ($O_2^{\cdot -}$), and approximately 1-2% of the $O_2$ consumed during normal physiological respiration is converted into this radical (Nohl, 1978).

The METC contains several redox centers that may leak electrons to $O_2$, serving as the primary source of $O_2^{\cdot -}$ production in most tissues. $O_2^{\cdot -}$ –producing sites and enzymes of the mitochondria have recently been discussed in a comprehensive review (Dröse, 2012). According to the current view, the main source of $O_2^{\cdot -}$ generation is complex I, from where ROS are released into the mitochondrial matrix and the intermembrane space, and the contributions of other complexes and sites seem to be relatively minor. However, others have observed that complex III is significant in ROS formation, and $O_2^{\cdot -}$ production by complex I

is markedly stimulated in the presence of succinate, indicating that a reverse electron flow is also involved (Bleier, 2012, Ishii, 2013).

The main consequences of oxidative stress forces are well-established, but the role of lipid peroxidation should be highlighted, especially in the mitochondria, where the highly reactive components of polyunsaturated fatty acids are particularly affected, in both the outer and inner membranes (Bindoli, 1988). This potentially harmful process proceeds by a ROS-mediated chain mechanism, when the multiple double bonds between fatty acids can rapidly be demolished, leading to membrane destruction. In the mitochondria, these vulnerable unsaturated moieties are found mainly in 1-saturated-2-unsaturated-diacylphospholipids and in cardiolipin, which is unsaturated at both positions (Pfeiffer, 1979).

*1.2. Biological gases*

Recent discoveries have radically altered the view of the roles of gases which had previously been considered biologically inert. Signaling functions have been demonstrated for nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide ($H_2S$), and research on gas mediators and derivatives has become a topic of interest (Dawson, 1994; Wang, 2003; Ryter, 2004; Wu, 2005; Rochette, 2008; Lamon., 2009; Motterlini, 2010). In this line, the activity or toxicity of the known gas mediators NO, CO and $H_2S$ is related to their tendency to react with biologically important molecules. It has become increasingly clear that these signaling agents take part in complex pathways, in which they are able to regulate numerous physiological processes, separately, or more often in antagonistic or synergistic ways (Gadhia, 2013; Rochette, 2013; Szabó, 2013).

*1.2.1. Methane ($CH_4$) as a novel biological gas*

Methane $CH_4$ was earlier commonly thought to be produced in the gastrointestinal (GI) tract exclusively by methanogenic bacterial fermentation, under strictly anaerobic conditions. This notion was challenged when various *in vitro* and *in vivo* studies revealed the possibility of nonmicrobial $CH_4$ formation in the mitochondria and eukaryote cells in both plants and animals. (Ghyczy 2008b; McLeod, 2008; Bruggemann, 2009; Messenger, 2009; Wishkerman, 2011). The biological role of methanogenesis in the mammalian physiology is largely unmapped, but it has recently been proposed that $CH_4$ may be a candidate of the gasotransmitter family (Wang, 2014).

*1.2.2. Nonbacterial generation of $CH_4$*

Recent *in vivo* studies have furnished persuasive experimental evidence of the nonbacterial generation of $CH_4$ in a variety of oxidoreductive stress conditions (Ghyczy, 2001, 2003, 2008; Keppler, 2006; Messenger, 2009). Various study designs have been

employed to identify the mechanistic components and the results suggest that the release of $CH_4$ may be a consequence of the production of ROS after transient $O_2$ deprivation (Ghyczy, 2008a; Keppler, 2008). Hypoxia is inseparable from a mitochondrial dysfunction, and ROS formation is especially pronounced in the inner mitochondrial membrane during the inhibition of cytochrome c oxidase activity (VanUffelen, 2008). More importantly, it has been shown in plants and eukaryotic cells that the $CH_4$-producing phenomenon can be mimicked by sodium azide ($NaN_3$) administration (Ghyczy, 2008; Whiskermann, 2011), when selective and stable inhibition of mitochondrial cytochrome c oxidase leads to chemical hypoxia with subsequent energy depletion (Bennett, 1996; Knyihár-Csillik, 1999; Szabados, 2004).

*1.2.3. $CH_4$ detection methods*

In consequence of its physicochemical properties, $CH_4$ traverses the mucosa and freely enters the splanchnic microcirculation. It is widely accepted that the bulk of the $CH_4$ produced is excreted via the lungs, and breath testing has therefore become a tool for the diagnosis of certain GI conditions in humans (Le Marchand, 1992; Lacy Costello, 2013). Nevertheless, $CH_4$ is distributed evenly across membrane barriers. The pulmonary route is therefore certainly not exclusive, and the production is reflected not only in the exhaled air, but also in its passage through body surfaces. Indeed, a recent study demonstrated the uniform release of $CH_4$ through the skin in healthy individuals (Nose, 2004). It follows that determination of the whole-body $CH_4$ output is required for an assessment of the magnitude of the release or clearance. To date, however, no studies have been reported in which the overall $CH_4$ generation was investigated or characterized *in vivo*.

In humans, the output of $CH_4$ or methyl group-containing compounds is usually measured in exhaled air samples by means of gas chromatography (GC) or GC-mass spectrometry (Lewitt, 2006; Ligor, 2008). Nevertheless, traditional methods of breath analysis have limitations and the risk of possible artefacts is high (Yu, 2004). A relatively new option is near-infrared diode laser-based photoacoustic spectroscopy (PAS), which has proven its relevance in life science applications (Cristescu, 2008). PAS is a special mode of optical absorption spectroscopy that is based on the conversion of absorbed light energy into acoustic waves. The technique is based on the thermal expansion of absorbing gas samples: the amplitude of the generated sound is directly proportional to the concentration of the absorbing gas component.

Despite the fact that numerous high-sensitivity PAS-based $CH_4$ sensors have been reported in the literature, their use for breath analysis is uncommon (Ngai, 2006). PAS provides high selectivity, sensitivity and reliability, and in addition, due to its robust design, it

is suitable for measurements outside the laboratory (Bozóki, 2011). Indeed, real-time atmospheric $CH_4$ measurements have recently been performed with PAS-based sensors (Rocha, 2012; Jahjah, 2014). Nevertheless, near-infrared diode lasers could be effectively used as gas sensors for medical applications due to their excellent stability, long lifetime and low cost.

*1.3. L-Alpha-glycerylphosphorylcholine (GPC)*

Phosphatidylcholine (PC) is an essential component of biomembranes and endogenous surface-coating substances, and it is well established that the main elements of ischemia/reperfusion (IR)-induced tissue injuries include lipid peroxidation and the loss of membrane-forming phospholipid bilayers (Volinsky, 2013). Likewise, it has been shown that a reduced PC content of the intestinal mucus plays significant roles in the development of inflammatory bowel diseases (Stremmel, 2012). Interestingly, a number of data suggest that choline-containing phospholipids, including PC, may function as anti-inflammatory substances under highly oxidizing IR conditions. Several studies have indicated that exogenous PC inhibits leukocyte accumulation (Erős, 2009; Hartmann, 2009) and the generation of inflammatory cytokines (Treede, 2009), and dietary PC administration has been demonstrated to provide protection against experimental neuroinflammation, arthritis and pleurisy (Erős, 2009; Hartmann, 2009; Tőkés, 2011) in rodents.

Furthermore, previous results have demonstrated that pretreatment with PC reduces the exhaled $CH_4$ concentration during an intestinal IR injury (Ghyczy, 2008a). In another *in vitro* study, PC metabolites with an alcoholic moiety in the molecule (i.e. choline, N,N-dimethylethanolamine and N-methylethanolamine) inhibited the ROS-producing activity of isolated leukocytes (Ghyczy, 2008b). Nevertheless, the specific mechanism of action of PC is still not known with certainty, and the question arises as to which of the moieties in the PC molecule are of critical significance in the reduction of the leukocyte responses and pro-inflammatory signal production. The PC molecule is composed of a choline head group and glycerophosphoric acid, with a variety of saturated and unsaturated fatty acids; given their potent bioactions, lipids may be pro-inflammatory or deactivate inflammatory pathway signaling *in vivo* (Bochkov, 2002; Nivala, 2013) and can possibly influence tissue damage. On the other hand, emulsions containing deacylated phospholipid derivatives do not induce endoplasmic reticulum stress or the activation of inflammatory pathway signaling (Nivala, 2013). Furthermore, GPC, a water-soluble, deacylated PC derivative, proved to be effective against lipid peroxidation and loss of the membrane function in oxidoreductive injuries (Onishchenko, 2008). GPC is an essential pro-phospholipid source for cell membranes and,

interestingly, the erythrocyte membrane contains predominantly this compound (Gross, 1994). It has been shown to act via cholinergic pathways (Drago, 1992; Sigala, 1992) and has a stimulant effect on the growth hormone response too (Ceda, 1992). GPC has been shown to be protective against membrane oxidation and can ameliorate the membrane function after traumatic injuries (Onischenko, 2008; Kidd, 2009).

## II. AIMS AND SCOPES

Our main objective was to investigate nonbacterial biotic methanogenesis and to shed light on the mechanistic details of the reaction.

**1.** In this framework, our first aim was to design a detection setup with which to investigate the *in vivo* dynamics of $CH_4$-producing phenomena specifically and reproducibly. Our primary goal was to determine the whole-body $CH_4$ release in living, unrestrained small animals.

**2.** An additional goal was to validate the $CH_4$ detection system in humans so as to obtain data comparable with those of other studies and methods used for clinical $CH_4$ breath testing.

**3.** We hypothesized that a dysfunction of the METC plays distinct roles in the biogenesis of $CH_4$. We set out to determine the *in vivo* $CH_4$ production profile after the induction of mitochondrial distress in rodents.

**4.** We hypothesized that the administration of the potentially methyl-group donor compound GPC might influence the nonbacterial $CH_4$ release.

**5.** We also hypothesized that the administration of GPC might afford protection in the form of antigen-independent inflammation accompanying an oxidoreductive imbalance. With this aim, we set out to investigate the effects of GPC in standardized animal models of antigen-independent forms of inflammation evoked by ischemia or irradiation.

## III. MATERIALS AND METHODS

### *3.1. Photoacoustic spectroscopy*

In our study we employed near-infrared diode laser-based PAS in cooperation with the Department of Optics and Quantum Electronics at the University of Szeged.

The instrument was calibrated with various gas mixtures prepared by the dilution of 1 vol % of $CH_4$ in synthetic air (Messer, Budapest, Hungary), and proved to have a dynamic range of 4 orders of magnitude; the minimum detectable concentration of the sensor was found to be 0.25 ppm ($3\sigma$), with an integration time of 12 s.

#### *3.1.1. Whole-body $CH_4$ analysis setup in rodents*

A new, purpose-built device was used to measure the gases emanating from small animals such as rodents as a function of time. Two sampling chambers were constructed for the animals, with regard to their average size and the air volume required for their normal respiration. The chambers and the gas-sampling system were made of glass and stainless steel, respectively, in order to minimize the adverse effects of adsorption-desorption. A 180 $cm^3$ sampling chamber was prepared for accumulation of the $CH_4$ emanating from a mouse, and another sampling chamber for measurements on rats (volume: 2510 $cm^3$). The chamber consisted of a glass cylinder and two metal plates, a fixed one and a removable one, with a gas outlet port situated on the fixed plate. The chamber included a removable stainless steel grid, ensuring that virtually the whole body surface of the animal remained uncovered. An animal was laid on the grid and placed into the chamber, and the cylinder tube was then closed with the removable plate.

In the event of continuous sampling, a constant gas concentration is established when the number of gas molecules released from the animal per unit time is equal to the number of gas molecules transferred by the volume of sampled gas per unit time. In other words, if the sampling flow rate is constant, a well-defined $CH_4$ emission corresponds to a given steady $CH_4$ concentration.

*3.1.2. Human study, participants and protocol*

$CH_4$ measurements in humans were performed at preset times (the same hour of the day) just before the first meal. The participants were subgrouped as males (n = 44) or females (n = 39), children (aged $\leq$ 20 years; n = 42) or adults (aged $\geq$ 21 years; n = 41). The $CH_4$ concentration of the exhaled air was measured. Participants were considered to be $CH_4$ producers if the exhaled $CH_4$ concentration exceeded 1 ppm.

The $CH_4$ concentration of the room air was determined and used as baseline in the calculations of the $CH_4$ emission. Afterwards, the subject was asked to breathe normally and the expired air was directed into a glass flask (volume: 200 $cm^3$). The optimal time for the attainment of a steady $CH_4$ concentration at a flow rate of 30 $cm^3$ $min^{-1}$ proved to be between 1 and 3 min.

*3.2. Experiments with small animals*

The experiments, on male Sprague-Dawley rats (220-300 g (bw)), and SKH-1 hairless mice (32-36 g bw) were performed in accordance with the National Institutes of Health guidelines on the handling and care of experimental animals. All the studies were approved by the Animal Welfare Committee of the University of Szeged. We set out different experimental model to mimic mitochondrial dysfunction-evoked oxido-reductive stress.

LPS-induced endotoxemia was performed int he mouse study, and $NaN_3$-induced chemical hypoxia, mesenteric ischemia/reperfusion and gamma irradiation-caused systemic inflmmation was performed in three different rat study.

*3.3. Biochemical measurements*

*3.3.1. ATP measurements*

A sample was taken from the liver, cooled in liquid nitrogen, and stored at -70 $^{o}$C. Afterwards, it was subsequently weighed, placed into a 3-fold volume of trichloroacetic acid (6% w/v), homogenized for 1 min, and centrifuged at 5000 g.

The ATP determinations were based on the measurement of luciferase chemiluminescence, using a luminometer (LUMAT LB 9507, Berthold Technologies, GmbH, Bad Wilbad, Germany). ATP levels were calculated with the aid of a standard ATP calibration curve (Sigma-Aldrich GmbH, Germany). The data were referred to the sample weights.

*3.3.2. Intestinal xanthine oxidoreductase (XOR) activity*

Small intestinal biopsies kept on ice were homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged at 4 $^{o}$C for 20 min at 24, 000 g and the supernatant was loaded into centrifugal concentrator tubes. The activity of XOR was determined in the ultrafiltered supernatant by fluorometric kinetic assay.

*3.3.3. Intestinal and lung tissue myeloperoxidase (MPO) activity*

Samples were homogenized with Tris-HCl buffer. 100 µl of homogenate supernatant was incubated for 5 min at 37 $^{o}$C. The reaction was started with 0.6 mM hydrogen peroxide ($H_2O_2$) dissolved in 0.75 ml of $K_3PO_4$ buffer) and was stopped after 5 min with 0.2 ml of $H_2SO_4$ (2 M) and the $H_2O_2$-dependent oxidation of tetramethylbenzidine was detected spectrophotometrically at 450 nm (UV-1601 spectrophotometer, Shimadzu, Kyoto, Japan). MPO levels were calculated via a calibration curve prepared with standard MPO (Sigma-Aldrich GmbH, Munich, Germany). The data were referred to the protein content.

*3.3.4. Intestinal superoxide (SOX) production*

The level of SOX production in freshly minced intestinal biopsy samples was assessed by a lucigenin-enhanced chemiluminescence assay. . Chemiluminescence was measured at room temperature in a liquid scintillation counter by using a single active photomultiplier positioned in out-of-coincidence mode, in the presence or absence of the SOX scavenger nitroblue tetrazolium (NBT; 20 µl). NBT-inhibited chemiluminescence was considered an index of intestinal SOX generation.

*3.4. Tissue injury analysis*

*3.4.1. Intravital videomicroscopy (IVM)*

The microcirculation of the liver surface was visualized by means of IVM, using a Zeiss Axiotech Vario 100HD microscope (100 W HBO mercury lamp, Acroplan 20x water immersion objective). Fluorescein isothiocyanate FITC; Sigma Chemicals, St. Louis, MO, USA, 0.2 ml iv) was used for the ex-vivo labeling of erythrocytes, and rhodamine-6G (Sigma, St. Louis, MO, USA, 0.2%, 0.1 ml i.v.) for the staining of PMN leukocytes.

*3.4.2. In vivo histology*

The dynamic structural changes of the liver were investigated by real-time laser scanning confocal endomicroscopy with an excitation wavelength of 488 nm, the emission being detected at 505 to 585 nm (FIVE1, Optiscan Pty Ltd, Notting Hill, Australia). The chosen areas were scanned in a raster pattern to construct a transverse optical section (1 scan per image, 1024 x 512 pixels and 475 x 475 μm per image). The optical slice thickness was 7 μm; the lateral and axial resolution was 0.7 μm. The liver architecture was examined *in vivo* following topical application of the fluorescent dye acridine orange (Sigma-Aldrich Inc, St. Louis, Missouri, USA).

*3.5. Statistical analysis*

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). Due to the non-Gaussian data distribution, nonparametric methods were used in the animal experiments, and thus Friedman repeated measures analysis of variance on ranks was applied within groups. Time-dependent differences from the baseline (0 min) for each group were assessed by Dunn's method, and differences between groups were analyzed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method for pairwise multiple comparisons. The Mann-Whitney U-probe was applied to assess the differences between human samples. In the Figures, median values and $75^{th}$ and $25^{th}$ percentiles are given. p values $< 0.05$ were considered significant.

## IV. RESULTS AND DISCUSSION

*4.1.PAS $CH_4$ detection*

In our studies, the detected $CH_4$ changes were reproducible; the instrument is highly specific for $CH_4$ and has a wide dynamic range, from levels of a few ppm to several thousands of ppm. In view of the long-term stability, control calibration is recommended only once a year. The range of application can easily be expanded by the addition of another light source to the system. PAS-based sensors have proved to be appropriate for multicomponent analyses of widely varying gas compositions (Besson, 2004; Scotoni, 2006; Hirschmann, 2008;

Hanyecz, 2010; Kosterev, 2010), indicating the potential for more complex gas analysis. The sampling chamber and the flow rate of the gas samples can be optimized for different experiments. Furthermore, the instrumentation can be used in acute or chronic experiments without exposing the animals to surgery, anesthesia or other trauma during the measurements.

*4.1.1. Whole-body $CH_4$ measurement in unrestrained rats*

In our study, the whole-body $CH_4$ generation of living, healthy rats was determined for the first time. In order to investigate the possible changes in $CH_4$ generation within a one-day interval, we included 4 healthy rats into the study and the $CH_4$ levels were recorded every second hour on two consecutive days. Data on the $CH_4$ metabolism of rats are lacking and no studies have been published where rats were considered to be $CH_4$ producers. We therefore analyzed the $CH_4$-producing capacity of these animals without exposure to any stress, before and after feeding, and also in their active and passive periods. We did not detect significant $CH_4$ emission (between 1.007 dppm/1000 $dm^2$ and 1.394 dppm/1000 $dm^2$) at baseline and did not find any changes in the whole-body $CH_4$ production regardless of the hour of the day. Thus, we regarded the result as proof of the lack of methanogenic flora in our laboratory rat strain.

*4.1.2. Human $CH_4$ measurements*

Even though the determination of the whole-body $CH_4$ release of anesthetized or unrestrained smaller animals provides more comprehensive data in a living system, the breath test is the method of choice with which to monitor human $CH_4$ production (Costello, 2013). In clinical practice, breath gas analysis has been widely used for the screening of patients with irritable bowel disease, where the observation of $CH_4$ concentration changes in relationship with other gaseous compounds can promote the diagnosis (Peled, 1987; Bratten, 2007). The $CH_4$ concentration in the breath is usually greater than 1 ppm in only 30-60% of humans (Costello, 2013). Regardless of sex and age, we found 21% producers, with a mean $CH_4$ concentration of 15.4 ppm. This proportion is broadly in line with recent findings based on GC measurements, where the average breath $CH_4$ concentration in adult $CH_4$ producers was 16.6 ppm (Levitt, 2006).

*4.1.3. Mitochondrial distress-associated $CH_4$ generation*

After the development of an appropriately specific and sensitive PAS-based detection system for $CH_4$, we investigated the functional role of mitochondrial electron transport in the biogenesis. We set out to determine the *in vivo* $CH_4$ production profile after the induction of inflammation and/or mitochondrial distress in rodents. Here we assumed that $CH_4$ excretion in the breath reflects intestinal bacterial fermentation plus an unknown and variable amount of

nonbacterial generation induced from target cells. We demonstrated that $CH_4$ generation is significantly increased in the groups exposed to a chronic $NaN_3$ challenge. The phenomenon proved to be independent from the methanogenic flora, since $CH_4$ emission was also elevated in the antibiotic-treated group. As endotoxemia and the specific inhibition of the mitochondrial complex IV lead to an increased $CH_4$ output, we assume that mitochondrial distress and the following inflammatory reaction might be the common denominator of $CH_4$ biogenesis.

### *4.2. The effects of GPC on $CH_4$ formation and inflammatory consequences of oxidoreductive stress*

We demonstrated the ability of GPC to effectively silence several inflammatory consequences linked to a reaction that might involve cellular or mitochondrial ROS generation. GPC is a centrally-acting cholinergic precursor which increases the tolerance to ischemic tissue damage (Onishchenko, 2008). Clinically, it is effective in cerebrovascular and neurodegenerative diseases (Parnetti, 2001; De Jesus Moreno Moreno, 2003), including ischemic stroke (Barbagallo Sangiorgi, 1994). More importantly, GPC can act as a choline source in various tissues (Bauernschmitt, 1993; Lee, 1993; Amenta, 1994).

As regards the above-mentioned studies, the ATP level of the liver was significantly decreased and the pro-oxidant molecules level were significantly increased in the untreated animals, and GPC treatment was proven to be protective against this effects. These findings may be linked to the membrane-conserving effect of GPC under oxidoreductive stress conditions. During reoxygenation, the components of the METC in the inner membrane are the main targets of ROS and reactive nitrogen species (RNS) (Raffaelo, 2011). If prolonged, such stress attacks lead to the otherwise reversible damage of the METC becoming irreversible, and functionless membranes and embedded proteins cannot synthesize ATP. Nevertheless, the METC- or membrane-protective action of GPC, including the inhibition of mitochondrial ROS or RNS formation, demands further, in-depth investigations.

## V. SUMMARY OF THE NEW FINDINGS

1. We have developed and used a new diagnostic procedure based on PAS for the real-time detection of $CH_4$ generation. With this technique, the daily $CH_4$ output can be determined and different stress-caused changes or treatment effects can be evaluated accurately and reproducibly.

2. The PAS-based spectroscopic method proved to be appropriate for reproducible and reliable breath $CH_4$ analysis in humans. The exhaled $CH_4$ concentration of a heterogeneous human population was monitored and the ratio of $CH_4$ producers was determined. There was no difference in the $CH_4$-producer ratio from the aspect of the sex, but significantly more $CH_4$ producers were found in the adult population as compared with those aged under 21 years.

3. The whole-body $CH_4$ emission profile of rodents was detected for the first time. The baseline $CH_4$ generation of rats and mice and also the changes in the $CH_4$ values after exposure to various chronic challenges inducing mitochondrial dysfunctions were determined.

4. The $CH_4$ level was significantly increased under oxidoreductive stress conditions, independently of the methanogenic bacterial production. This phenomenon might be an alarm signal linked to the earlier phase of oxidoreductive stress states.

5. The administration of GPC terminated stress-associated $CH_4$ generation in rodents, and alleviated the inflammatory consequences of acute oxidoreductive stress. The pro-oxidant enzyme activity and SOX level were reduced and the liver ATP content was maintained on GPC treatment. Accordingly, GPC supplementation may provide protection against hypoxia or irradiation-caused stress conditions and might be a promising therapeutic agent with which to influence such events.

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