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My contribution to original papers

Paper I

Performing of oxygraphic measurements. Performing of western blot analysis. Designing of primers and assistance in performing PCR reactions. Collection and analysis of all data. Writing of the Material and Methods and Results sections of the paper and preparing most of the Figures and Table 2. of the manuscript.

Paper II

Performing of oxygraphic measurements. Collection and analysis of all data. Writing of the Methods and Results and part of Introduction and Discussion sections of the manuscript. Prepearing of all figures and the table of the manuscript.

Paper III

Performing of oxygraphic measurements. Collection all data and analysis of all data, except for transmission electron microscopy. Writing of the manuscript and preparing of Figures 1 and 3 and the Table.

ABBREVIATIONS

AG	atrophic gastritis
ADP	adenosine 5`-diphosphate
AK	adenylate kinase
AMP	adenosine monophosphate
ANT	adenine nucleotide translocase
AP_5A	diadenosine pentaphosphate
ATP	adenosine 5`-triphosphate
ATPase	adenosine triphosphatase
ATR	atractyloside
BB-CK	brain type creatine kinase
CK	creatine kinase
COX	cytochrome oxidase
Cr	creatine
Cyt c	ctochrome c
FAD	flavin adenine dinucleotide
$FADH_2$	1, 5-dihydro-flavin adenine nucleotide
FCCP	carbonylcyanide-p-trifluorometoxy-phenolhydrazone
GC	gastric cancer
GM	gastric mucosa
GSH	glutathione
IAK	index adenylate kinase
ICK	index creatine kinase
LDH	lactate dehydrogenase
MOM	mitochondrial outer membrane
MtAK	mitochondrial adenylate kinase
MtCK	mitochondrial creatine kinase
MtDNA	mitochondrial DNA
NADH	dihydronicotinamide adenine dinucleotide
OXPHOS	oxidative phosphorylation
PA	pernicious anemia
PG I	pepsinogen I
PG II	pepsinogen II
PCr	phosphocreatine
RC	respiratory chain
ROS	reactive oxygen species
SDH	succinate dehydrogenase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
S-PGI	serum pepsinogen I
S-PGII	serum pepsinogen II
UQ	ubiquinone (coenzyme Q)
UQH_2	ubiquinol – reduced UQ

INTRODUCTION

Nowadays, gastric cancer (GC) represents one of the most challenging tumors due to the fact that its diagnosis is often late and, in the advanced stage, the therapeutic options are scarce with a consequent high rate of mortality. In fact, although a reduction in global incidence for GC is reported, it remains the second cause of cancer-related death (Ferlay et al., 2010; Forman and Burley, 2006). In general, survival rates of GC have been low. The EUROCARE study estimated average European survival for the cases diagnosed in the period 1995–1999 to be around 46% at 1 year and 25% at 5 years after diagnosis (Sant et al., 2009). These results indicate that the knowledge and data concerning the development of GC are still unclear. Additionally, the knowledge of precursor lesions for the development of GC could contribute to anticipating GC diagnosis at an early stage when surgery or chemotherapy offers a better prognosis.

Based on extensive cohort studies conducted in Columbia, as well as on data gathered in Estonia, Finland and Japan, Pelayo Correa proposed a paradigm of gastric carcinogenesis that has become known as Correa`s cascade. According to this, the biological model of gastric carcinogenesis can be displayed as an inflammation-atrophy-metaplasia-dysplasia-carcinoma sequence (Correa, 1992) that is based on three different intermingled processes. Firstly, chronic active inflammation caused by *Helicobacter pylori* (*H. pylori*) creates the background for geno- and phenotypic alterations. Secondly, disruption of the balance between apoptosis and cell proliferation results in mucosal atrophy. Thirdly, progressive loss of differentiation favors establishment of intestinal metaplasia characterized by replacement of intestine-type glands for normal glands (Correa, 2004).

It is conceivable that transition of the normal mucosal cell phenotype towards the cancer cell phenotype may be accompanied or even underlied by specific alterations in cellular energy metabolism, particularly at the level of mitochondrial functions. On the one hand, mitochondria support the function and viability of the cell by converting the energy released from substrate oxidation into adenosine triphosphate (ATP). On the other hand, mitochondria represent the key organelles capable of initiating and controlling apoptotic cell death. Both mentioned functions are largely disturbed in cancer cells, which was first noted by Otto Warburg who proposed that development of cancer is causally related to suppression of oxidative phosphorylation (OXPHOS) and activation of aerobic glycolysis (Warburg, 1956). Later on, many studies have proved the correctness of the Warburg hypothesis. It has been proposed that alterations in the respiratory chain (RC) of mitochondria trigger reactive oxygen species (ROS) production, which in turn accelerates and aggravates the impairment of the mitochondrial structure. In parallel, the ROS dependent signalling pathways become activated, which eventually result in such bioenergetic rearrangements that favor cancer development (reviewed in Seppet et al., 2009). Controversially, other investigations revealed contradictory modifications with

the upregulation of OXPHOS components and a larger dependency of cancer cells on oxidative energy substrates for anabolism and energy production (Jose et al., 2011; Moreno-Sánchez et al., 2007).

Today, however, there is only very limited information concerning the bioenergetic function of the mitochondria in the human normal gastric mucosa (GM) and GC *in situ*. In the present research, we used saponin treated (permeabilized) gastrobiopsy specimens of the antrum and corpus mucosa for studies of OXPHOS system in human GM. Our study addresses mainly the function of mitochondrial RC in three stages (chronic inflammation, atrophic gastritis (AG) and GC) of inflammation→atrophy→carcinoma sequence, to find out the possible markers of early changes in GM leading to GC.

Besides, the evidence confirming possible participation of creatine kinase (CK) and adenylate kinase (AK) isoforms in the energy transfer systems in human GM cells is still lacking. In many cells with intermittently high and fluctuating energy demands (cardiac muscle, brain and spermatozoa) the mitochondria and ATPases are linked to each other by specialized phosphotransfer systems mediated by different CK) and AK isoforms. Thus we aimed to characterize energy transfer systems in human GM.

REVIEW OF LITERATURE

1. Gastric mucosa and energy metabolism

1.1. Function of gastric mucosal cells

Anatomically the human stomach can be divided into four sections each of which has different cells and functions. The first section is the cardia and the second section is the fundus. The third and the main section is the body or corpus of the stomach. The fourth, the distal section, is the antrum, that ends at the pylorus.

The gastric epithelium is folded to form glands, which are the distinctive feature of the GM. The gastric glands are: (a) cardiac glands (b) fundus or oxyntic glands and (c) antral or pyloric glands. The cardiac glands, few in number, occur close to the cardiac orifice. The fundus glands are found in the corpus and fundus of the stomach. In the corpus of the stomach, the major differentiated glandular cell types are parietal cells secreting HCl, zymogen (chief) cells secreting pepsinogen I (PG I) and pepsinogen II (PG II), and surface epithelial/folveolar/mucus neck cells secreting mucus glycoprotein and PG I and PG II (Samloff and Liebman, 1973; Samloff and Taggart, 1987). In humans the parietal cells secrete also the intrinsic factor. The most important endocrine cells in the corpus epithelium are histamine releasing enterochromaffin-like (ECL)-cells and somatostatin releasing D-cells. In the antrum glands parietal cells and ECL-cells are absent. The two main endocrine cell types in this region are gastrin releasing G-cells and D-cells. The GM in the antrum region produces PG II (Samloff and Liebman, 1973; Samloff and Taggart, 1987).

More specifically, the epithelium is composed of tubular invaginations known as the gastric units. Each unit is self-renewing and contains a single multipotent class of stem cells continuously fueling the unit with new cells. The multipotent stem cell is located in the upper third of the gastric gland and in the region called the isthmus. Some cells migrate upwards, becoming mucus secreting surface epithelial cells. They differentiate and migrate for 3 days and then they are exfoliated into the lumen. Other cells within the proliferative zone differentiate into parietal, chief or ECL cells and subpopulations migrate towards the base of the unit, surviving 50–190 days (Björkholm et al., 2003; Dockray, 1999). The interactions between different epithelial cell types influence partly proliferation and differentiation. The presence of active mature parietal cells seems to be very important to maintain the normal cellular proliferation and commitment differentiation program of the gastric epithelium (Karam, 1995). Loss of parietal cells also causes reprogramming of the zymogenic chief cell lineage so that the genes that are normally expressed only in mucous neck cells, such as spasmolytic polypeptide/TFF2, are expressed at high levels in cells at the base. Proliferation is increased and occurs more basally in the unit. The pattern of basal proliferation and coexpression of neck and zymogenic cell genes is similar to the histological pattern in the normal antrum and

pylorus (pseudopyloric metaplasia) (Mills and Shivdasani, 2011). In addition pyloric antral hormone gastrin 17 regulates only acid secretion, but also the proliferation of gastric epithelial cells (Dockray, 1999) and the relative numbers of different cell populations in the epithelium, thereby influencing secretory capacities (Friis-Hansen et al., 1998).

1.2. Oxidative phosphorylation: respiratory chain and mechanism of ATP production in mitochondria

Adenosine triphosphate (ATP) is the universal energy currency of living cells and as such is used to drive numerous energy-consuming reactions, e.g., biosynthesis of proteins and nucleic acids, mechanical motility, transport through membranes, regulatory networks, and nerve conduction. Most of the ATP in animal cells is generated by mitochondria primarily through OXPHOS – a process that couples cellular respiration and ATP synthesis and is capable of producing significantly more ATP per molecule of substrate than glycolysis in reactions completely dependent on the availability of oxygen. Structurally, mitochondria are organelles enclosed by two very distinct membranes: an outer membrane (MOM), which is moderately selective, and an inner membrane, which is protein rich and highly selective. The system of OXPHOS consists of four respiratory enzyme complexes of the electron transport chain (also called RC, OXPHOS complexes I–IV) and ATP synthase (complex V), which are arranged in a specific orientation in the mitochondrial inner membrane. Two membrane soluble electron carriers ubiquinone (UQ, co-enzyme Q) and small protein cytochrome c (Cyt c), are also involved in electron transport. The respiratory complexes are NADH;UQ oxidoreductase (complex I), succinate dehydrogenase also succinate:quinone oxidoreductase (complex II), ubiquinol:Cyt c oxidoreductase, also cytochrome bc1, (complex III), and cytochrome c oxidase (COX) (complex IV).

Generally, the electrons originating from oxidation of organic nutrients such as glucose are used to reduce of nicotinamide adenine dinucleotide (NAD) to generate NADH (mostly in the Kreb`s cycle, also called tricarboxyic acid cycle, located in the mitochondrial matrix). Electrons from NADH are passed along a series of respiratory enzyme complexes, and the energy released by this electron transfer is used to pump protons across the membrane, specifically at the complexes I, III and IV, so that the energy is stored in the form of the electrochemical gradient, so called protonmotive force (Δp). Electrons are ultimately transferred to molecular oxygen, with its complete reduction to water (respiration). As respiration proceeds, the resulting increase in the protonmotive force will drive ADP phosohorylation in the ATP synthase complex (De Oliveira et al., 2012; Saraste, 1999).

The mitochondrial RC constitutes the important intracellular source of ROS in most tissues. In various respiratory complexes leak electrons to oxygen, pro-

ducing primarily the superoxide anion ($O_2^{-\bullet}$). The relative contribution of every site to overall $O_2^{-\bullet}$ production varies from organ to organ and also depends on whether mitochondria are actively respiring (State 3) or the RC is highly reduced (Turrens, 2003). Therefore the retarded flow of electrons in RC is involved in the production of ROS. When ROS formation exceeds defense capacity, dangerous ROS attack all biomolecules (oxidative stress), acutely reducing the activity of RC chain enzymes, but chronically impairing the nuclear and MtDNA (Seppet et al., 2009; Turrens, 2003) In inflammation, increased nitrogen oxide (NO) production reinforces oxidative stress in the mitochondria via reversible inhibition of RC complexes and formation of peroxynitrite from O_2^- and NO (Beltrán et al., 2000; Borutaite et al., 2000; Boveris et al., 2002; Clementi et al., 1998; Masci et al., 2008; Moncada, 2000). In connection to the ROS production, changes in the activity of different RC complexes modify cell cycle activity, cell growth and proliferation, apoptosis, cell transformation and cancer (Barrientos and Moraes, 1999; Galli et al., 2003; Hüttemann et al., 2012; Klimova and Chandel, 2008; Simonnet et al., 2002; Stefanatos and Sanz, 2011).

The system of OXPHOS in the GM has not been profoundly studied. In the earlier papers the OXPHOS in the GM was mainly studied in the context of formation of stress ulcers (Martin et al., 1987, 1982; Sato et al., 1978). A major objective of the present study was to investigate whether *in situ*, the respiratory activity of RC complexes is affected in diseased human GM.

1.2.1. Complex I

Complex I (NADH:ubiquinone oxidoreductase E.C:1.6.5.3.) is the major entry point for electrons to the RC in mammalian mitochondria (Hirst, 2010). It catalyzes the transfer of two electrons from NADH to UQ, in parallel with the translocation of four protons (recent data suggest three protons (Wikström and Hummer, 2012) across the inner mirochondrial membrane. This activity contributes about 40% of the protonmotive force coupled to mitochondrial ATP production. In mammals, complex I is the largest complex in the mitochondrial OXPHOS system. It contains 45 subunits, and results in apparent molecular mass of about 1 MDa. The complex contains multiple redox centers: one flavin mononucleotide, nine iron–sulfur (FeS)-centers, covalently bound lipid and protein bound semiquinones (Brandt, 2006; Carroll et al., 2006; Hirst, 2010; Ohnishi, 1998; Walker, 1992; Yagi and Matsuno-Yagi, 2003). Seven subunits of the complex are encoded by the MtDNA, 38 are encoded by nuclear genes are located in different chromosomes. Complex I is a regulable pacemaker of the mitochondrial respiratory function (Hüttemann et al., 2007; Remacle et al., 2008; Scacco et al., 2003; Yadava et al., 2008). Preserving the normal functioning of complex I is fundamental to secure mitochondrial ATP production and its supply to the cells. Complex I is also a major site of cellular $O_2^{-\bullet}$ production. Namely, during a sequential, stepwise electron transfer process there

occurs direct electron leakage to oxygen. This happens at different redox sites of the enzyme (Cadenas and Davies, 2000; Fato et al., 2008; Grivennikova and Vinogradov, 2006; Hirst, 2010). Complex I is involved in apoptosis (Fontaine and Bernardi, 1999) and in age-related functional decline (Gadaleta et al., 1998; Papa, 1996; Ventura et al., 2002). Various hormones, neurotransmitters and cell growth factors regulate, through the activation of cellular signal transduction networks, the expression and functional activity of complex I (De Rasmo et al., 2011; Franko et al., 2008; Hüttemann et al., 2007; Robinson, 1998; Scacco et al., 2000). Complex I dysfunction has, indeed, been found to be associated with several human diseases. Therefore, studies reflecting the respiratory function of complex I in case of gastric diseases may provide important information about the functioning of the OXPHOS system in the GM cells.

1.2.2. Complex II

Complex II (succinate dehydrogenase (SDH) also known as succinate:quinone oxidoreductase) catalyzes the flavin adenine dinucleotide (FAD)-dependent, two-electron oxidation of succinate to fumarate, coupled with the reduction of UQ to UQH_2. Complex II is a component of a Kreb`s cycle and it converts succinate to fumarate. The electrons from succinate oxidation directly contribute to OXPHOS. Complex II does not translocate protons, and therefore it only feeds electrons to the RC (Hägerhäll, 1997; Rutter et al., 2010). Mammalian complex II is a heterotetrameric membrane-protein complex, encoded only by the nuclear genome.

The active site domain is faces the matrix side of the mitochondrial inner membrane. It consists of a flavoprotein, in which the covalently bound FAD is located, and an iron sulfur protein. The membrane domain has two subunits, which contain heme groups (Sun et al., 2005; Yankovskaya et al., 2003).

Two UQ binding sites have been identified in SDH complexes in mammals and in *Escherichia coli* (Sun et al., 2005; Yankovskaya et al., 2003). Ubiquinone reduction occurs in two stepwise single electron reactions, in contrast to the two-electron reduction of FAD. But the architecture of enzyme redox centers is arranged in a way that prevents ROS production at the FAD site (Brand, 2010; Yankovskaya et al., 2003). Heme could be involved in the prevention of ROS formation during electron transfer from FAD to UQ by acting as a capacitor in case of high electron flux. The presence of the heme center may prevent ROS formation with a reverse electron flow from UQH_2, minimizing the level of semiquinone (Kim et al., 2012a; Yankovskaya et al., 2003). In partnership with UQ, the SDH will represent a crucial antioxidant enzyme in the mitochondria, controlling the superoxide scavenging activity of RC.

Mutations in the four SDH genes (SDHA, B, C and D) have been reported, resulting in strikingly diverse clinical presentations, including cancer (reviewed in Brière et al., 2005; Lemarie and Grimm, 2011; Rutter et al., 2010). Recent data demonstrate that, a subset (7% to 10%) of gastric gastrointestinal stromal

tumors (GISTs) is notable for the immunohistochemical loss of succinate SDH subunit B (SDHB), which signals the loss of function of the SDH complex (Miettinen et al., 2013). The present study aims to Investigate of SDH respiratory activity in GM of patients with chronic gastritis, AG and GC.

1.2.3. Complex III

Complex III (ubiquinol:Cyt c oxidoreductase, cytochrome bc1, EC 1.10.2.2) catalyzes transfer of electrons from UQH2 to diffusible soluble Cyt c, and this reaction is coupled to transmembrane proton translocation. Mammalian complex III is a dimer. Each monomer consists of 11 different subunits but the protein contains only three subunits with redox prosthetic groups (reviewed in Saraste 1999, Kim et al., 2012).

Complex III couples the electron delivery redox reaction to the generation of a proton gradient across the membrane by a mechanism known as the Q cycle, during which, for each transported pair of electrons, two protons are uptaken from the mitochondrial matrix and four protons are translocated into the innermembrane space and two molecules of Cyt c are reduced (reviewed in Saraste 1999; Kim et al. 2012; de Oliveira et al. 2012).

Complex III is the primary site for the net ROS generation in mitochondria during the oxidation of the substrates of complex I (Chen et al., 2003) but its contribution to total ROS production may vary in different tissues (Turrens, 2003). Complex III is the dominant site, because ROS products are released mainly into interamembrane space, and are therefore directed away from the strong antioxidant defense of the matrix (Chen et al., 2003; St-Pierre et al., 2002; Turrens et al., 1985). ROS acts as an important second messenger in intracellular signaling cascades in GM cells in spite of its notoriety for causing cell damage. ROS can also induce and maintain the oncogenic phenotype of cells, deprive of cancer's c of oxidative stress or antioxidants to block the progression of malignant transformation (Kim et al., 2012b).

In our study the activity of RC complexes I+III+IV and II+III+IV will be determined in case of different diseases of GM including GC, to indicate also the role of complex III in gastric diseases.

1.2.4. Complex IV

Complex IV (Cytohrome c oxidase or COX, also ferrocytochrome c: oxygen oxidoreductase, EC 1.9.3.1) is a terminal component of the RC. The enzyme catalyzes the transfer of four electrons from four reduced Cyt c to oxygen, reducing it to two water molecules. The electron transfer is electrogenic and is coupled to proton translocation across the inner mitochondrial membrane. The overall reaction is

$$4\ \text{Cyt. } c^{2+} + O_2 + 8\ H^+_{in} \rightarrow 4\ \text{Cyt. } c^{3+} + 2\ H_2O + 4\ H^+_{out}$$

The mammalian complex IV has 13 subunits and it contains several redox centers (Tsukihara et al., 1996). Three major subunits are coded by MtDNA and form the functional core of the enzyme (Van Kuilenburg et al., 1991). According to the results purified from beef heart and crystallized. We know that COX exists as a homo-dimer (Tsukihara et al., 1996).

Since COX retains all partially reduced intermediates until full reduction is achieved, the production of ROS is negligible (Turrens, 2003). Interestingly, at the very same site oxygen binds to the COX, which is also able to bind cyanide, carbon monooxide and NO, all of which are respiration inhibitors (Nicholls and Ferguson, 2002). Nitric oxide particularly has been shown to be of interest, since it is an ubiquitous signal molecule that is able to reversibly inhibit mitochondrial respiration (Antunes et al., 2007; Moncada, 2000; Sarti et al., 2012).

Thus we aimed to reveal how the activity of complex IV is affected in case of human gastric diseases.

1.2.5. ATP synthase

The human mitochondrial ATP synthase (F_1F_o-ATPase/synthase), or complex V (EC 3.6.3.14) is the fifth multi subunit OXPHOS complex. The F_1F_o-ATP synthase is a miniature engine composed of two well defined opposing rotary motors F_1 and F_o which are physically connected by two stalks. The F_1 motor catalyses ATP synthesis or hydrolysis by a rotary mechanism. The F_o motor catalyses proton translocation across the membrane (Von Ballmoos et al. 2009; Watt et al. 2010; Jonckheere et al. 2012). Each 360° rotation of F_o produces three ATP molecules in the F_1 domain, while the bioenergetic cost for the enzyme to produce one ATP is 2.7 protons (Watt et al. 2010).

Recent findings support the contribution of the ATP synthase to the execution of cell death. This function is exerted by control of ROS production by the RC. Santamaria et al showed that the downregulation of the ATP synthase, and thus that of OXPHOS, is part of the molecular strategy adapted by rat hepatoma cells to avoid ROS-mediated cell death (Santamaría et al., 2006). Isidoro and colleagues showed depressed expression of the β-catalytic subunit of the mitochondrial ATP synthase in human biopsies taken from gastric adenocarcinoma (Isidoro et al., 2004).

1.3. Energy transfer systems and their coupling to OXPHOS

In general, in intact cells increased ADP production in ATPase reaction must result in elevation of mitochondrial respiration rate. Precise coupling of spatially separated intracellular ATP consumption and ATP production is fundamental to the bioenergetics of a living organism. For optimal function the energy-rich phosphoryl groups are produced and delivered to energy-consuming sites at the rate corresponding to ATPase velocity. In turn the products of ATP

hydrolysis: ADP, Pi, and H^+, are removed in order to avoid product inhibition of the enzymes (Dzeja et al., 2000; Saks et al., 1994). Also the ATP produced by mitochondria cannot accumulate in the intermembrane space (space between mitochondrial outer and inner membranes), because it would inhibit export of ATP from the mitochondrial matrix by locking the adenine nucleotide translocator (ANT) (Mannella et al., 2001). Produced by the ATPase reactions, ADP apparently cannot diffuse freely and serve as a feedback signal to the ATP-regenerating reactions, as abundant catalytically active CK, AK and glycolytic enzymes, residing throughout a cell, would process large portion of the ADP produced by ATPase reactions (Dzeja et al., 2000; Saks et al., 1994). However, energy transfer by diffusional exchange of adenine nucleotides is kinetically and thermodynamically inefficient since it requires a significant concentration gradient. Now is known that ATP utilizing and ATP producing sites (OXPHOS and the Krebs cycle in the mitochondria and glycolysis in the cytosol) communicate with each other using enzymatic phosphotransfer networks that facilitate transfer of energy rich phosphoryl groups without significant fluctuations of cytosolic adenine nucleotide concentrations. Rapidly equilibrating enzymatic systems of creatine and adenylate kinases can operate by the vectorial ligand conduction mechanism, providing a conduit for energy rich phosphoryls (Dzeja and Terzic, 2003; Saks et al., 1994).

The basic mechanism of regulation energy fluxes trough the network connecting mitocondrial OXPHOS and cytosolic ATPases is functional coupling with microcompartmelization (Saks et al., 2004, 1994). In the mitochondrial microcompartment of the system the enzyme mitohondrial isoform (MtCK or AK2) functions in the direction of PCr synthesis or ADP generation, respectively. The functional coupling of MtCK or AK2 to ANT in turn facilitates the transport of the ADP into the mitochondria. Increased ADP concentration in the matrix stimulates OXPHOS and the respiration rate of the mitochondria increases. Next, the changes in the ADP/ATP and PCr/creatine concentrations in the cytosol are conducted via cytocolic isoforms (MM-CK, BB-CK, MB-CK or AK, see below) to the microcompartment near the ATPase where the cytosolic CK/AK isoforms work in the direction of ATP regeneration. In that compartment ADP is quickly rephosphorylated by the cytosolic isoform functionally coupled to ATPase (Dzeja and Terzic, 1998; Saks et al., 2004, 1994). One important consequence of microcompartmentalization is that the turnover of ATP and ADP molecules within the microcompartments between adjacent enzymes may be much faster than their diffusion within the cell volume – this may have an important consequence for the feedback signal in the cells.

It has been observed that short-term inhibition of proton pumps (H^+–K^+-ATPase) with omeprazole or H_2-receptor antagonists (ranitidine) suppresses mitochondrial activity in the corpus of the human stomach (Hui et al., 1989). This blocks mitochondrial processes and abolishes the H^+-pumping activity of the gastric glands (Rong et al., 1998). Besides, alterations in the secretory activity of parietal cells occur together with changes in mitochondrial

morphology (Jiang et al., 2002; Spicer et al., 2000). This implies that GM cells must possess intracellular mechanisms enabling exact regulation of mitochondrial ATP production in accordance with altered ATP utilization in secretory processes. The CK and AK energy transfer systems in the GM are poorly characterized. Marked CK and AK activities have been detected in cultured GM cells (He et al., 2004; Rong et al., 1998; Sistermans et al., 1995). In the parietal cells BB-CK is strongly expressed (Sistermans et al., 1995; Wold et al., 1981) and colocalizes and couples functionally to H-K-ATPase to effectively provide ATP for proton pumping (Sistermans et al., 1995). The expression of uMtCK has been found in the human intestine (jejunum and ileum) (Payne and Strauss, 1994) but not yet in the stomach. On the other hand, exchange between adenine nucleotides mediated by AK is markedly activated in the conditions of generation of ATP by mitochondria (Rong et al., 1998). Although these data point to the possible participation of CK and AK isoforms in the energy transfer systems in human GM cells, the evidence confirming this is still lacking.

1.3.1. Creatine kinase system

The CK (ATP:creatine N-phosphoryl transferase EC 2.7.3.2) system consists of a small family of isoenzymes, which catalyse reversible exchange of energy rich phosphate groups between phosphocreatine (PCr) and ADP through the reaction: ADP + PCr + $H^+ \leftrightarrow$ ATP + creatine. In mammals two cytosolic isoforms ubiquitous brain type B-CK and sarcomeric muscle type M-CK, as well as two mitochondrial subunit isoforms, ubiquitous uMtCK and sarcomeric sMtCK, are synthesized in a tissue specific manner. The cytosolic subunits form ezymatically active homodimers (MM-CK and BB-CK) or heterodimers (MB-CK) (Wallimann et al., 1992). The uMtCK and sMtCK localized in the outer side of the mitochondrial inner membrane are encoded by two separate nuclear genes and both form octameric molecules which are composed of four active and stable dimers (Haas and Strauss, 1990; Schlegel et al., 1988). The cytosolic components of the CK shuttle, M-CK and B-CK, are expressed coordinatedly in their tissue distribution with mitochondrial CKs, sMtCK and uMtCK, respectively. The uMtCk RNA is expressed in many tissues similar to B-CK but the expression of the isoforms does not predict their functional role and the functional coupling of OXPHOS or ATPases (Payne and Strauss, 1994; Ventura-Clapier et al., 1998).

The role of CK in energy metabolism may involve several functions. First, a "temporal energy buffer function" *i.e*. regeneration of ATP at the expense of PCr, when requirements of the cell are fast-growing (e.g. contraction in fast twitch skeletal muscles) and energy supply from mitochondria is insufficient). Second, facilitated intracellular energy transduction (CK shuttle hypothesis or "spatial energy buffer", described above) *i.e.* linking of mitochondrial ATP generation to cytosolic sites of ATP utilization via mitochondrial and cytosolic

CK isoenzymes (Bessman and Geiger, 1981; Saks et al., 1994; Wallimann et al., 1992). Thus CK plays a particularly important role in tissues with large and fluctuating energy demands like the muscle and the brain. Third, the function of the CK system is to avoid an increase of intracellular [free ADP] and thereby prevent product inhibition of cellular ATPases (Wallimann et al., 1992). The ATP requirement of the GM also differs many times between the resting and the secreting states. Fourth is the proton buffering function. Since the CK reaction in direction of ATP regeneration utilizes not only ADP but also protons (H^+), an intimate functional coupling of CK to ATPases prevents the global or local acidification of cells, which are hydrolyzing high amounts of ATP within a short period of time (Wallimann et al., 1992). Interestingly, the gastric parietal cells contain a large amount of BB-CK that is not associated with recognizable cellular structures. Unbound BB-CK activity may mitigate extreme fluctuations in intracellular pH and ATP/ADP ratios that occur in energy demanding proton transport process (Sistermans et al., 1995).

1.3.2. Adenylate kinase system

Adenylate kinase (AK; EC 2.7.4.3) is ubiquitous enzyme that catalyzes the reaction ATP + AMP ↔ 2ADP. By providing for utilization both β-and γ-phosphoryls of ATP, AK doubles the energetic potential of the ATP molecule (Dzeja and Terzic, 1998).

So far eight AK isoforms have been cloned, which exhibit different levels of tissue expression and with distinct intracellular distribution. Tissues with high energy demand, such as the brain, heart and skeletal muscle are rich in AK1, the major enzyme isoform (Dzeja et al., 1998; Inouye et al., 1999; Janssen et al., 2000). AK2 is located mainly in the mitochondrial intermembrane space as well as in cytosol. AK2 has been detected in human heart, liver kidney and lungs (Khoo and Russell, 1972; Noma et al., 1998). Adenylate kinase 3 is an ubiquitous GTP:AMP phoaphotransferase, (EC. 2.7.4.10), catalyzes the reaction GTP + AMP ↔ GDP + ADP) and is present exclusively in the mitochondrial matrix of all tissues except for red blood cells (Noma, 2005; Noma et al., 2001). The main task of AK3 is to generate GDP and ADP using GTP produced in the Krebs cycle. AK4, AK5 and AK6, AK7 and AK8, all of these enzymes have different kinetic parameters that may indicate their contribution to adenosine nucleotide homoeostasis in different microenvironments and have specific cellular functions (Dzeja et al., 2002; Fernandez-Gonzalez et al., 2009; Liu et al., 2009; Noma et al., 2001; Panayiotou et al., 2010, 2011; Ren et al., 2005; Solaroli et al., 2009; Van Rompay et al., 1999; Yoneda et al., 1998).

The presence of the AK isoforms participating in energy transport systems in the human GM has not been studied.

2. Diseases of gastric mucosa and energy metabolism

2.1. Chronic gastritis

Chronic gastritis is an inflammatory condition of the GM characterized by elementary lesions whose types, extent and distribution are related to their etiology and are modulated by host responses and environmental factors. Infection with *H. pylori* is the most common cause of chronic active gastritis; chemical agents, autoimmunity, and other infections are the cause of very small proportion of chronic, usually non-active gastritis.

According to the Sydney System, chronic gastritis is recognized by lymphocytic and plasma cell infiltration (mononuclear infiltration) of superficial or deep mucosa. Chronic active gastritis is characterized by the co-existence of polymorphonuclear (presence of specific neutrophil infiltration) inflammation besides mononuclear inflammation (Misiewicz et al., 1991). The phenomenon of active gastritis is considered to reflect the reaction of the host against *H. pylori* infection and to be strongly associated with the risk of progression of gastritis assuming an atrophic pattern, which is also related to the acquiring cytotoxic *H. pylori* strains.

Chronic gastritis is a very common disease among Estonians, with peculiarities of behavior in the antrum and corpus mucosa. Gastritis of any grade was found in the antrum and/or the body in 96–98% subjects representing an Estonian urban population (Maaroos et al., 1990; Villako et al., 1990). The high prevalence rate of gastritis can be explained with a very high prevalence *H. pylori* infection in the Estonian population, which is according to serological evaluation approximately 90% in adults (Vorobjova et al., 1994, 2008).

H. pylori induces inflammation of the GM by a number of factors, such as the vacuolating cytotoxin gene A (vacA), genes localized in a cag pathogenicity island and lipopolysaccharide. Immunological studies by Vorobjova et al. (Vorobjova et al., 1999, 2000) demonstrated high prevalence (63%) of antibodies to cytotoxity-associated protein gene A (CagA) protein (a marker of cag pathogenity island) in a random sample of adult population and among schoolchildren (46%) in Estonia. The virulence of *H. pylori* is also highly expressed at the gene level as 87% of patients with different gastric diseases presented the cagA gene that correlated strongly with the highly cytotoxic vacuolating cytotoxin gene A (vacA) signal sequence type s1a (Andreson et al., 2002). A long term-follow up study of Maaroos and colleagues (Maaroos et al., 1999) showed that anti-CagA positivity was significantly associated with the activity but not severity of chronic gastritis in the antrum and corpus GM.

Increased production of ROS is a hallmark of inflammation. Excess production of ROS, exerting cytotoxic effects on gastric epithelial cells via lipid peroxidation, membrane damage, and reduction of cellular glutathione (GSH) content (Davies et al., 1994; Jung et al., 2001; Naito and Yoshikawa, 2002;

Santra et al., 2000; Xia and Talley, 2001) is a major component of the inflammatory response of the tissue. It is exists evidence that mitochondria may play an important role in development of gastric inflammation not only as being a source and target of the ROS but also as the organelles specifically attacked by *H. pylori*.

Previously it has been demonstrated that in cultured gastric cell lines the N-terminal 34 kDa fragment of vacA cytotoxin of *H. pylori* directly permeabilizes MOM (Galmiche and Rassow, 2010; Galmiche et al., 2000). This process is associated with depolarization and fragmentation of mitochondrial membranes with decreased rate of mitochondrial respiration, diminished membrane potential and suppressed ATP synthesis (Ashktorab et al., 2004; Kimura et al., 1999). Additionally, is noted increased production of ROS, NO and ammonia, all of which secondarily exert cyto- and mitochondriotoxic effects have been described (Jung et al., 2001; Kubota et al., 2004; Xia and Talley, 2001). Impairment of MOM by vacA causes also the release of Cyt c (Galmiche et al., 2000; Maeda et al., 2002) and activation of apoptosis (Ashktorab et al., 2004; Maeda et al., 2002). Mitochondrial contribution to inflammation may vary between the corpus and antral GM, because the former contains more mitochondria than the latter (Martin et al., 1987; Sato et al., 1978). For example the *H.pylori*-linked inflammation upregulates the superoxide dismutase mitocondrial isoform in the antrum but not in the corpus (Broide et al., 1996; Farkas et al., 2003; Götz et al., 1997, 1996). Yet it is unclear whether these diversities affect regulation and intracellular organization of energy metabolism in the GM

2.2. Atrophic gastritis

Is known that almost in half of uncured cases the chronic gastritis caused by *H. pylori* transforms into gastric corpus AG with variable grades (Valle et al., 1996). Chronic AG is an inflammatory condition characterized by the loss of gastric glandular structures, which are replaced by connective tissue (non-metaplastic atrophy) or by glandular structures inappropriate for the location (metaplastic atrophy) (Rugge et al., 2002). Epidemologically, AG in the gastric corpus is strongly associated with the intestinal subtype of adenocarcinoma (according to Laurén's classification). For subjects with AG only in the corpus region, the relative risk of GC is 2–5 (depending on the grade of AG) compared to subjects with normal GM. (Vauhkonen et al., 2006). The risk of gastric neoplasias rises exponentially with increasing grade and extent of AG (and intestinal metaplasia) in the antrum and corpus and is particularly high in patients with the advanced disease (Correa, 1992; Sipponen et al., 1985; Vauhkonen et al., 2006). Severe AG in both the antrum and corpus (severe multifocal AG) increases the risk of GC 45–90 times compared with cancer risk in subjects with a normal healthy stomach, or in subjects with non-atrophic *H. pylori* gastritis (Sipponen et al., 1985). Atrophic gastritis of corpus can be diag-

nosed and evaluated by serological screening using surrogate markers of gastric function (serum level of PG I or PG I/PG II ratio) or by gastroscopy and histology (Agréus et al., 2012).

In an Estonian adult population sample AG was more common in the corpus than in the antrum (Villako et al., 1990, Maaroos et al 1999, Vorobjova et al., 2001). The long-term follow up showed that the progression of atrophy in the antrum GM, and particularly in the corpus GM, is associated with CagA seropositive *H pylori* infection. The CagA positivity was also more closely associated with activity of gastritis and development of AG in the corpus than in the antrum GM (Maaroos et al., 1999, Vorobjova et al., 2008). However, in cases of AG and GC, the immune response to *H. pylori* (IgG level) declined significantly in comparison with chronic non-atrophic gastritis (Vorobjova et al., 2006). Interestingly, the weak response of *H pylori* antibody is a risk factor for GC (Tulinius et al., 2001, Yamaji et al., 2002). Further, the GC risk is the highest in subjects with AG in whom the numbers of bacteria are the lowest, or *H. pylori* is totally absent or has disappeared (Vauhkonen et al., 2006).

The end stage of corpus AGis associated with pernicious anemia (PA). PA is a macrocytic anemia that is caused due to vitamin B_{12} deficiency, as a result of intrinsic factor deficiency. The deficiency of intrinsic factor is a consequence of the presence of AG, which results in the destruction of the oxyntic mucosa, and thus the loss of parietal cells, which normally produce hydrochloric acid as well as intrinsic factor. PA is considered an autoimmune disorder due to the frequent presence of gastric autoantibodies directed against intrinsic factor, as well as against parietal cells (Lahner and Annibale, 2009; Toh et al., 1997).

Concerning AG, it is clear that drastic changes in GM *i.e.* intestinal metaplasia, should develop alterations not only in the tissue content of mitochondria, but also in their functional parameters, which in turn, may promote transition of normal mucosa into the cascade of carcinogenesis. However, the mitochondrial function in these conditions is yet unknown. An electron microscopic study by Yin and colleagues (Yin et al., 2003a, 2003b) reveals that in case of AG the number of mitochondria is decreased and the amount of mitochondrial abnormalities and damages is increased compared with a normal GM. Accordingly, a decreased capacity of OXPHOS might be expected. It is also conceivable that atrophy of the glandular mucosa imposes qualitative alterations upon RC and ATP synthesis, thereby inducing transition from a normal GM to the cancer tissue.

2.3. Gastric cancer

Based on many earlier studies, the morphogenesis of the intestinal subtype of GC is considered to be a gradual and stepwise dedifferentiation of intestinal epithelium in to autonomously growing tumors that form glandular structures resembling those in adenocarcinomas of the gut in general. The characteristics of ‘intestinalized epithelium’ by morphology, immunohistochemistry and

molecular biology can be recognized in intestinal type gastric adenocarcinomas to varying extents, degrees and combinations. The diffuse subtype of GC (according to Laurén`s classification) is not connected with the intestinal-type epithelium, AG or intestinal metaplasia. So far, *H. pylori* gastritis is the only universal precursor condition for diffuse type of GC (Vauhkonen et al., 2006).

Extensive amount of information about bioenergetics in cancer cells reveals the metabolic reprogramming as a hallmark of cancer (Jose et al., 2011). It is known that the metabolic shift from OXPHOS to aerobic glycolysis (Warburg effect), tolerance to hypoxic microenvironment, ability to control ROS level and avoidance of apoptosis are the characteristics of cancer cells, greatly contributing to viability, autonomous growth, migration and chemoresistance (Seppet et al., 2009). However, comparison of different cancer cell lines and excised tumors revealed a variety of cell's bioenergetic signatures (from highly glycolytic to partial OXPHOS phenotypes and even to enhanced OXPHOS phenotypes). Differences in the OXPHOS status originate from variability in metabolic reprogramming among cancer cells and from the contributions of oncogenes, tumor microenvironment (availability of substrates and oxygen) and proliferative activity as well as alterations in mitochondrial biogenesis (Jose et al., 2011; Moreno-Sánchez et al., 2009; Zu and Guppy, 2004; Weinhouse, 1956). Zu and Guppy analysed numerous studies and showed that aerobic glycolysis is not inherent to cancer but is rather a consequence of hypoxia (Zu and Guppy, 2004). Furthermore, the functional OXPHOS system appears necessary for maintaining tumorigenicity in many types of cancer (Fogal et al., 2010; Gough et al., 2009; Moreno-Sánchez et al., 2007; Rodríguez-Enríquez et al., 2000; Sonveaux et al., 2008; Tomitsuka et al., 2010). At the same time, the role of OXPHOS in maintaining the viability of human cancer cells is still largely unclear and, therefore, the investigation of OXPHOS in GC cells was an aim of our study.

In many cancer cells, the OXPHOS are characterized by defective RC complexes I and III and decreased β-F_1-ATPase (Boitier et al., 1995; Bonora et al., 2006; Bravo et al., 2004; Capuano et al., 1996; Cuezva et al., 2004, 2002; Green and Grover, 2000; Isidoro et al., 2004; Kuhnt et al., 2007; Petros et al., 2005; Ray and Ray, 1997; Simonnet et al., 2003, 2002). Moreover, the type of mitochondrial impairment appears to determine the clinical phenotype (Simonnet et al., 2003, 2002). Accordingly, benign oncocytomas are characterized by impaired complex I with enhanced expression of other RC complexes and matrix enzymes, together with upregulation of mitochondrial tissue content. These changes evidently compensate for the insufficient complex I. In contrast, malignant renal tumors exhibit downregulation of all RC complexes and F_1-ATPase activity, in correlation with increased tumor aggressiveness and avoidance of apoptosis (Simonnet et al., 2003, 2002). Also, the functioning and regulation of mitochondrial RC contributes to cellular ROS homeostasis and hindrance of electron flow in the RC leads to production of ROS (De Oliveira et

al., 2012; Seppet et al., 2009). The respiratory function of RC complexes in the human GC cells is not known.

Isidoro and colleagues showed that the expression level of the β subunit of F_1F_0-ATP synthase in gastric adenocarcinomas was significantly reduced and the expression of the marker of glycolysis glyceraldehyde-3-phosphate dehydrogenase was increased (Isidoro et al., 2004). The usage of spotted cDNA microarrays identified a proliferative signature of the intestinal type GC (Boussioutas et al., 2003). In the light of these observations, one may suggest decreased OXPHOS in the GC cells.

AIMS OF THE STUDY

The aims of the study were the following:

1. To characterize AK and CK energy transfer systems in the human gastric mucosa
2. To assess the respiratory capacity of OXPHOS in the non-atrophic gastric mucosa of the antrum and corpus of patients with active and non-active chronic gastritis.
3. To characterize qualitative differences in mitochondrial respiratory function between the antrum and corpus mucosa in the case of non-atrophic gastritis.
4. To characterize comparatively the function of OXPHOS in the corpus mucosa of patients with corpus dominant atrophic gastritis with pernicious anemia, and in patients with non-atrophic gastric corpus mucosa in relation with S-PGI level.
5. To comparatively characterize the function of OXPHOS in human gastric corpus mucosa in patients with gastric cancer and patients with non-atrophic gastric mucosa as well as in human gastric cancer cell lines MKN28 and MKN45.

MATERIAL AND METHODS

1. Patients

Table 1. summarizes the number and sex distribution of the patients involved to studies. For the study of OXPHOS in human diseased non-atrophic GM (paper I), 40 consecutive patients from southern Estonia who underwent upper gastrointestinal endoscopy for epigastric complaints were recruited. None of these subjects exhibited corpus mucosa atrophy, nor had they received nonsteroidal anti-inflammatory drugs, H^+-pump inhibitors, or antibiotics to treat their illness.

Twelve patients with pernicious anemia (PA) were included in the second study as a group of patients with atrophic corpus gastritis (paper II). The criteria for the diagnosis of PA were following: macrolytic anemia, appearance of parietal cell antibodies, and low serum vitamin B_{12} and folic acid. The diagnosis of corpus AG was based on low serum pepsinogen I (S-PGI) level and histological confirmation of gastric body mucosal atrophy. The control group was formed of patients using the same criteria as for patients in Paper I.

Six patients with gastric non-cardial adenocarcinoma, located in the corpus compartment of the stomach, and referred to the department of oncological surgery for diagnostic examination, were included in the third study (paper III) as the cancer group. Patients with no signs of mucosa atrophy and active chronic inflammation in the corpus were selected to represent the control group.

Table 1. Number of patients used in the study. Values in years of age are presented as mean ± SE.

	Study group		Control group	
	Male	Female	Male	Female
	Mean age		Mean age	
Paper I	23	17	–	
	60 ± 2	64 ± 3	–	
Paper II	5	7	7	5
	67 ± 4		66 ± 3	
Paper III	3	3	5	5
	68 ± 3		64 ± 3	

2. Endoscopy and biopsy sampling

Mucosal biopsies were obtained from the anterior and posterior walls of the medial part of the corpus and from the antrum (2 cm above the pylorus from the anterior and posterior walls of the gastric antrum) as recommended in the Sidney System (Misiewicz et al, 1991; Dixon et al., 1996). In patients with GC, mucosal biopsies were taken from the visually detectable cancer tissue and from

the visually normal tissue surrounding it. In subsequent assessment these biopsies were grouped and referred to as „Adjacent" and „Cancer", respectively. One part of each biopsy specimen was used to determine the histopathology of GM and the presence of *H. pylori*, for which these specimens were fixed overnight in neutral buffered formalin and embedded in paraffin. Tissue sections were stained for morphological and *H. pylori* examination by hematoxylin and eosin and modified Giemsa methods. The presence and severity of chronic gastritis, activity of gastritis, atrophy, and intestinal metaplasia were graded according to the Sidney System, from 0 (no changes) through 1 (mild) and 2 (moderate) to 3 (severe changes) (Misiewicz et al., 1991; Dixon et al., 1996). Infiltration of lymphocytes indicated the chronic status of inflammation, and abundant presence of mononuclear cells marked an active chronic process. The amount of *H. pylori* in the mucosa was estimated semiquantitatively by microscopic counting as described earlier (Maaroos et al., 1990; Peetsalu et al., 1991). Another part of the mucosa specimens was placed immediately in ice-cold solution was A and used for studies of mitochondrial function.

The gastric biopsies were carried out in accordance with the European Communities Council Directive 86/609/EEC and with Declaration of Helsinki (1997). Written informed consent was obtained from all patients, and the Tartu University Ethics Committee approved the studies.

3. Blood samples and laboratory tests

Basal blood samples for measurements of serum pepsinogen I (S-PGI) were drawn after an overnight fast. Samples for S-PGI were collected into serum tubes. The serum tubes were centrifuged at 1500 g for 10 min and the samples were stored at −70°C until analyzed. S-PGI was determined using specific c enzyme immunosorbent assay (EIA) tests (Pepsinogen-I EIA Test Kit; Biohit, Helsinki, Finland), and the procedure was performed on a microwell plate according the manufacturer's instructions. Biohit, Finland provided all technical equipment required for the EIA techniques.

4. Cell cultures

The human GC cell line MKN28 was derived from moderately differentiated tubular adenocarcinoma and the cell line MKN45 from undifferentiated adenocarcinoma of medullary type (Motoyama et al., 1986). The cells were grown in flasks, in the presence of RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine and 1% antibiotic solution (Invitrogen) at 37° C and 5% CO_2. In 70–80% cell confluency, cells were removed from the plastic simply by shaking the flask or by using quick Trypsin treatment and thereafter used for cell physiology experiments.

5. Solutions

Composition of the solutions used for preparation of permeabilized mucosal tissue for oxygraphy.

Solution A contained (in mM) CaK2EGTA, 2.77, K2EGTA, 7.23, MgCl2, 6.56, DTT, 0.5, K-MES, 50, imidazole, 20, taurine, 20, Na2ATP, 5.3, phosphocreatine, 15, pH 7.1 adjusted at 25°C

Solution B contained (in mM): 2.77 CaK_2EGTA, 7.23 K_2EGTA, 1.38 $MgCl_2$, 0.5 DTT, 100 K-Mes, 20 imidazole, 20 taurine, 3 K_2HPO_4 and 5 mg/ml bovine serum albumin, pH 7.1 adjusted at 25°C.

Mitomed solution contained (in mM): 110 sucrose, 0.5 EGTA, 60 K-lactobionate, 3 $MgCl_2$, 20 taurine, 0.5 dithiothreitol, 20 HEPES, pH 7.1 adjusted at 25°C.

6. Preparation of the permeabilized mucosal tissue

The mucosal tissue samples were cut into 1 x 1.5-mm pieces in the ice-cold solution A. With the use of thin needles, the tissue pieces were gently stretched in all directions to mechanically separate the cells from each other. The tissue was then transferred into vessels with ice-cold solution A containing 50 μg/ml saponin and incubated at mild stirring for 30 min for permeabilization of the plasmalemma due to removal of cholesterol from the cell membrane by saponin. The indicated conditions were found to be optimal for maintaining mitochondrial function inside the permeabilized cells. The permeabilized mucosal tissues were then washed three times in solution B to remove all metabolites.

7. Preparation of permeabilized cells

The cells were centrifuged at 200 g for 5 min at room temperature, the supernatant (growth medium) was decanted and sedimented cells were resuspended in 5 ml Mitomed solution and centrifuged again as above. After removing the supernatant, the cells were incubated in shaking conditions for 15 min at room temperature in 6 ml Mitomed solution containing 65 μg/ml saponin. After that the cells were centrifuged and resuspended with Mitomed, this washing step was repeated twice, and the final pellet was resuspended in Mitomed and used immediately for experiments.

8. Analysis of the function of the respiratory chain

The saponin-treated tissues were incubated in solution B in the chamber (volume 1.5–2.5 ml) of the oxygraph (Rank Brothers, England or Oroboros, Paar KG, Austria), equipped with the Clark electrode, assuming the solubility of

oxygen in the medium to be 215 nM O_2/ml (Kuznetsov et al., 1996). Figure 1 demonstrates the original recording of the oxygraphic experiment. After the registration of basal respiration rate (V_0) in nonphosphorylating conditions in the presence of 10 mM glutamate and 2 mM malate (state 2 respiration), 2 mM ADP was added to monitor the maximum rate of NADH-linked ADP-dependent respiration (state 3 respiration) (V_{Glut}), followed by successive additions of 10 μM rotenone to inhibit the complex I, 10 mM succinate to activate $FADH_2$-linked ADP-dependent respiration (V_{Succ}), 0.1 mM atractyloside (ATR) to assess respiratory control by ANT (V_{Atr}), 10 μM antimycin A to inhibit the electron flow from the complex II to Cyt c, 0.5 mM N,N,N,′N′-tetramethyl-p-phenylenediamine (TMPD) with 2 mM ascorbate to activate Cyt c oxidase (COX), and 5 mM NaN_3 to quantify COX activity (V_{COX}) as the NaN_3-sensitive portion of respiration. Antimycin-sensitive respiration in the presence of atractyloside was considered to measure the respiration related to proton leak.

The permeabilized MKN cells were incubated in an oxygraph chamber in the Mitomed solution at 25 ºC containing 2 mg/ml fatty acid free bovine serum albumin (BSA), 0.2 μM free Ca^{2+} detected fluorimetrically (Eimre et al., 2008), in the presence of 10 mM glutamate and 2 mM malate as the respiratory substrates.

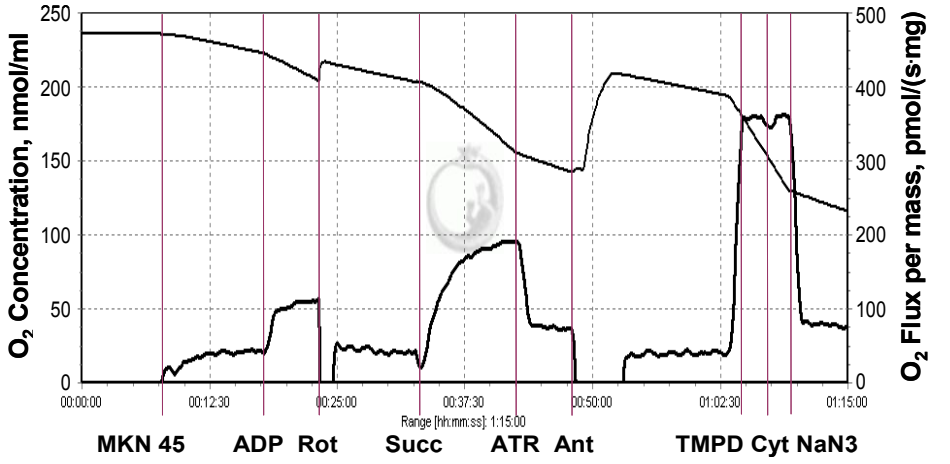

Figure 1. Original recording of respirometric investigation in MKN45 cells. Upper curve – O_2 concentration; lower curve – O_2 flux per mass. Additions: MKN45 – MKN45 cells treated with saponin, ADP – 2 mM MgADP, Rot – 10 μM rotenone, Succ – 10 mM succinate, ATR – 0.1 mM atractyloside, AntA – 10 μM antimycin A, TMPD – 0.5 mM TMPD with 2 mM ascorbate, Cyt c – 8 μM cytochrome c, and NaN3 – 5 mM NaN_3.

9. Analysis of coupling OXPHOS to MtCK and MtAK

The coupling OXPHOS to MtCK was estimated by two types of experiments. One of the protocols comprises also the analysis of the coupled reaction between ANT and MtAK.

Protocol 1. After the registration of V_0 in nonphosphorylating mitochondria in solution B with glutamate plus malate, 50 μM ATP was added to produce a minimum amount of endogenous ADP to stimulate mitochondria. AMP (2 mM) was then added to activate the coupled reaction of AK2 with ANT followed by addition of 0.2 mM diadenosine pentaphosphate (AP_5A) to inhibit AK (Seppet et al., 2005, Fig 3). To assess the strength of the functional coupling independently of mitochondrial content in individual GM preparations, activation of respiration by AMP was normalized for the respiratory rate registered before the addition of AMP, thus producing the relative index (I_{AK}): [$I_{AK} = (V_{AMP} - V_{ATP})/V_{ATP}$]. After AP_5A, 20 mM creatine (Cr) was added from solid to induce the coupling between MtCK and ANT, the efficiency of which was measured as CK index (I_{CK}): [$I_{CK} = (V_{Cr} - V_{AP5A})/V_{AP5A}$]. Thereafter, 2 mM ADP was added for maximum activation of respiration (V_{ADP}). Then 0.1 mM ATR was added to monitor control by ANT over OXPHOS. The maximum capacity of the RC was estimated in the presence of 2 μM carbonyl cyanide *p* trifluoromethoxyphenylhydrazone (FCCP) (V_{FCCP}). In the same protocol, the intactness of MOM was controlled by the addition of 8 μM Cyt c (Saks et al., 1998, Seppet et al*.,* 2005).

Protocol 2. The V_{O2} vs. [ADP] relationships were examined in the solution B supplemented with 10 mM glutamate, 2 mM malate, 4 U/ml hexokinase, and 11 mM glucose in the presence and absence of 20 mM creatine, and the corresponding apparent K_m and V_{max} values were calculated.

10. PCR Reactions

After preparation and reverse transcription of total RNA, the PCR reactions were performed. The forward and reverse oligonucleotide primer pairs matching the sequences of human AK1, AK2, brain-type CK (B-CK), and uMtCK, were designed by the Primer Express software (Applied Biosystems). PCR amplification was performed using the QuantiTect SYBR Green PCR Kit (Qiagen) with the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The thermal profile for real time-PCR (RT-PCR) comprised initially 15 min at 94°C to activate HotStarTaq DNA polymerase. This was followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30-s steps. After the 35th cycle, the amplified cDNAs were separated in a 1.7% agarose gel to verify amplicones by size.

11. SDS-PAGE and immunoblotting

Thirty-five micrograms of total protein in homogenates was separated by standard 12% SDS-PAGE and electrotransferred by semidry blotting (Hoefel Pharmacia Biotech, San Francisco, CA) on a nitrocellulose membrane (Shleicher and Schüell, Dassel, Germany), according to the manufacturer's instructions. The membranes were blocked with 4% fat-free milk powder in T-TBS (20 mM Tris·HCl pH 7.5, 150 mM NaCl, 0.05% Tween 20) overnight at 4°C, incubated for 15 min at room temperature and washed for 4 x 5 min with T-TBS. The membranes were then incubated for 1 h with uMtCK rabbit immunesera (1:2,000 dilution in a blocking buffer) or with affinity-purified chicken anti-B-CK IgY (1:500 dilution in a blocking buffer) at room temperature (Schlattner et al., 2002). For detecting AKs, the membranes were incubated for 1 h at room temperature with rabbit polyclonal antibodies against AK1 (H-90; Santa Cruz Biotechnology, Santa Cruz, CA) or AK2 (H-65; Santa Cruz Biotechnology) (dilution 1:500 in a blocking buffer), washed for 4 × 5 min in T-TBS and incubated for 1 h with the peroxidase-coupled secondary antibody, either goat anti-rabbit IgG (Nordic, Lausanne, Switzerland) (1:1,000 dilution in a blocking buffer) or rabbit anti-chicken IgY (Jackson ImmunoResearch, West Grove, PA) (1:3,000 dilution in a blocking buffer), and were finally washed for 4 × 5 min with T-TBS. The blots were developed with the enhanced chemiluminescence substrate (Amersham, Buckinghamshire, UK) and exposed to an X-ray film.

12. Statistical analysis

Where appropriate, one-way ANOVA with Bonferroni post-test and unpaired or paired Student's t test were used to analyze the differences between the groups Correlation analysis was performed by Pearson's test. A P value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

1. Function of energy transfer systems in diseased gastric mucosa (Paper I)

Our study shows (Fig. 2) that the antral and corpus GM expressed B-CK, uMtCK, AK1, and AK2 in mRNA and protein. The presence of mitochondrial and cytosolic isoforms of CK and AK proteins points to existence of intracellular energy transfer systems linking OXPHOS and ATPases.

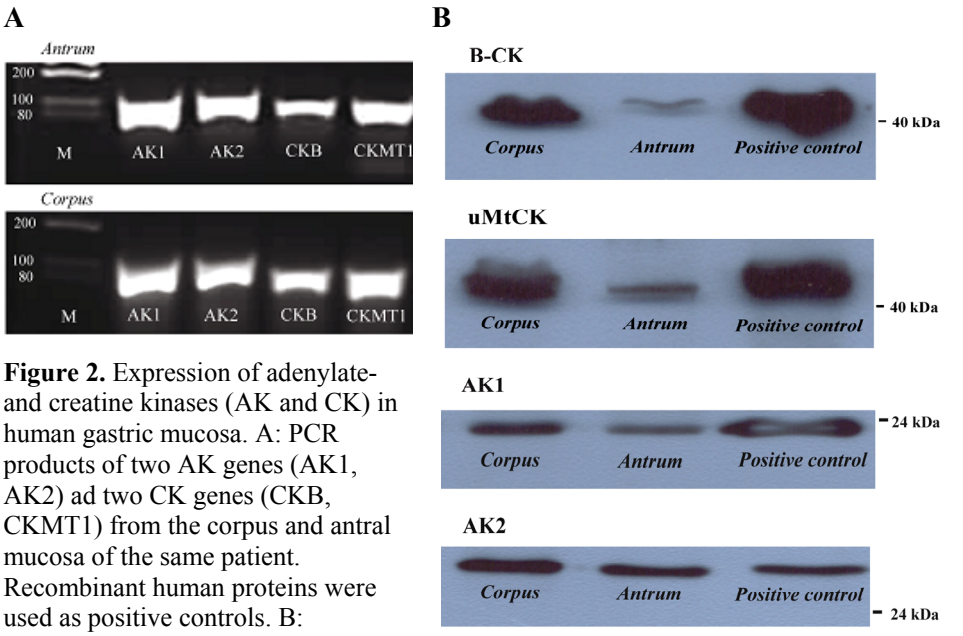

Figure 2. Expression of adenylate- and creatine kinases (AK and CK) in human gastric mucosa. A: PCR products of two AK genes (AK1, AK2) ad two CK genes (CKB, CKMT1) from the corpus and antral mucosa of the same patient. Recombinant human proteins were used as positive controls. B: Immunoblots tested with antibodies against AK1, AK2, ubiquitous MtCK (uMtCK) and B-CK. For AKs, proteins from the cytosolic and mitochondrial fractions of the rat heart were used as positive controls for AK1 and AK2, respectively.

Since the mitochondrial isoensyme of AK was expressed in GM, we tested the presence of functional coupling between AK2 and ANT. As presented in Fig. 3, addition of 2mM AMP strongly stimulates respiration in the presence of minute concentrations of ATP (50 μM). Besides, it triggered the functional coupling between AK2 and OXPHOS, thereby increasing local [ADP] near ANT, which results in elevated respiration rate. Participation of AK in this process was confirmed by the effect of AK inhibitor AP_5A. Namely, the respiration

decreases after addition of the compound down to the level before AMP addition (Fig. 3). The extent of stimulation by AMP was expressed as I_{AK}. The I_{AK}, an index independent of tissue mitochondrial content, was smaller for the antrum (0.52 ± 0.04, n = 27) than for the corpus (0.65 ± 0.05, n = 27) ($P < 0.05$). This difference may indicate a much tighter coupling between AK2 and ANT or more active AK2 in the corpus GM.

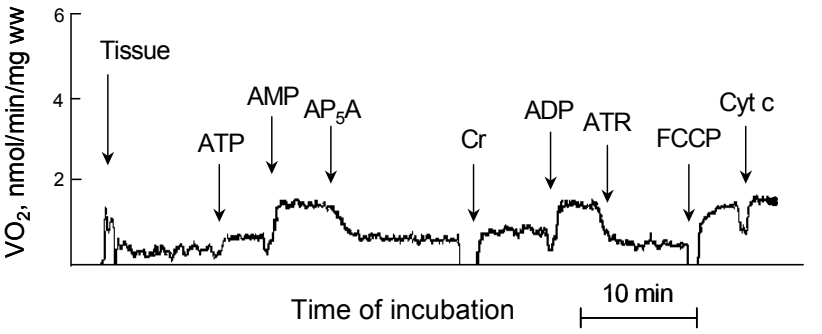

Figure 3. Original recording of a respirometric investigation of the coupling of AK and CK to oxidative phosphorylation in a permeabilized mucosal specimen from the gastric corpus. V· O2 was measured in the presence of 10 mM malate and 2 mM glutamate as the respiratory substrates. Additions: Tissue, permeabilized mucosal tissue 2–5 mg ww; 50 μM ATP; 2 mM AMP; 0.2 mM diadenosine pentaphosphate (AP_5A); 20 mM creatine (Cr); 2 mM ADP; 0.1 mM atractyloside (ATR); 2 μM FCCP; and 8 μM cytochrome c (Cyt c).

The functional coupling between uMtCK and OXPHOS was tested using two protocols. In the first protocol (Fig. 3) the inclusion of 20 mM creatine after addition of AP_5A reactivates respiration. The I_{CK} value in the corpus was 0.50 ± 0.09 and in the antrum 0.37 ± 0.06, although without statistical significance a tendency was similar as in case of I_{AK}. Here, two principally different mechanisms can account for the creatine effect.

The first mechanism (Model I) assumes fast equilibrium between ADP concentrations in the intermembrane space, cytosol, and medium to take place because of the simultaneous action of cytosolic CK and ATPases. According to this model, creatine added to the medium in the presence of ATP, may activate cytosolic CK and the CK bound to the endoplasmic reticulum and mitochondria, which increases [ADP] in the cytoplasm/medium. The increase of [ADP] stimulates OXPHOS as ADP diffuses through MOM and is transported into the matrix.

Alternatively (Model II), creatine can stimulate local production of ADP near ANT by MtCK at the expense of mitochondrially produced ATP. This

mechanism is known as the functional coupling between ANT and MtCK. The functional coupling allows establishment of higher local [ADP] in the microcompartment between MtCK and ANT than in the cytoplasm surrounding mitochondria (Saks et al., 2004).

To discriminate between these two models, another set of experiments was performed. In these experiments the interaction between MtCK and ANT was estimated from the effects of creatine on the V_{O2} vs [ADP] relationships in the presence of excess amounts of hexokinase and glucose. This system completely utilizes the ATP generated in the mitochondria when it is available for hexokinase and allows clamping of [ADP] at desired levels in experimental settings. Linearization of V_{O2} vs. [ADP] dependencies in the double-reciprocal coordinates provides the apparent K_m values for ADP to be 107.7 ± 6.6 μM and 93.5 ± 11.2 μM (Fig. 4A) in the antral and corpus mucosa, respectively. Our study also demonstrates (Fig. 4B) that creatine did not exert on V_{max}. In the presence of creatine, the apparent K_m, decreases markedly in both studied tissues (Fig. 4A)

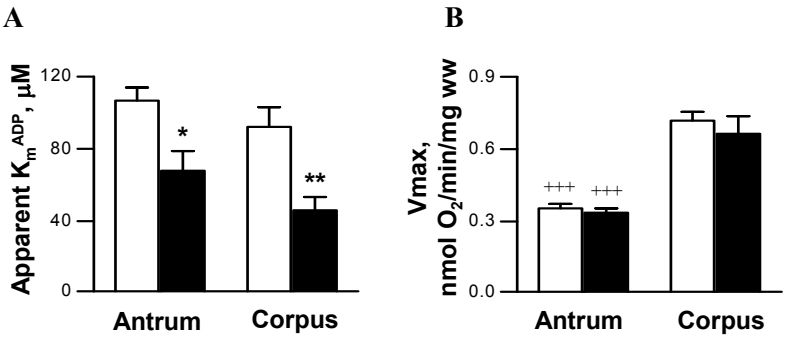

Figure 4. The effect of 20 mM creatine on the kinetics of regulation of respiration by exogenous ADP. A: apparent K_m. B: V_{max} in permeabilized gastric mucosa. B and C: filled bars with creatine; open bars without creatine; $n = 6$ for each group $^*P < 0.05$, $^{**}P < 0.01$ compared to assessment without creatine. +++ $P < 0.001$ vs. corpus.

The decrease in the apparent K_m value also confirms an effective functional coupling between OXPHOS and MtCK. It is highly unlikely (considering the presence of high activity of hexokinase in the medium), that extra ADP, which stimulates OXPHOS after addition of creatine, originates from the BB-CK reaction. Hence, there must have been an ATP pool inside the mitochondria (in the intermembrane space) inaccessible to hexokinase. In this mitochondrial microcompartment the ATP pool was converted to ADP by MtCK reaction in the presence of creatin. After that, ADP was transported into mitochondria by ANT for stimulate respiration. Because local [ADP] near ANT builds up above corresponding submaximal [ADP] in the medium/cytoplasm in this process, [ADP] vs. V_{O2} relationship changed toward diminished apparent K_m as com-

pared with its value without creatine. This type of the creatine effect on mitochondrial respiration is characteristic of the functional coupling between ANT and MtCK [Model II described above (Saks et al., 2004)].

These findings in combination with earlier results (Sistermans et al., 1995) allow us now to envisage the entire system of CK-phosphotransfer in antrum and corpus GM comprising all three necessary stages. First, the synthesis of PCr in mitochondria, second the transfer of energy-rich phosphoryl groups from PCr via cytosolic BB-CK, and third, the regeneration of ATP in the vicinity of ATPases because of the coupling of BB-CK to ATPases.

Additionally, we found that mitochondria in the GM specimens exhibited impaired (leaky) MOM. Intactness of MOM was assessed by monitoring the effect of excess Cyt c added to the uncoupled mitochondria (Fig. 3). In the case of leaky MOM, the mitochondria would have lost Cyt c, and its replenishment would have increased the respiration rate (Saks et al., 1998). In fact, practically all GM specimens exhibited a smaller or larger stimulation of respiration by Cyt c. For a whole group of specimens studied (n = 54 from 27 patients), the mean Cyt c effect was 17 ± 2%, with minimum and maximum individual values of 0 and 42%, respectively. It was also found that the prevalence of the Cyt c effect > 20% was twice larger for the preparations of the antral GM (14 of 27 patients, 52%) than for the preparations of the corpus GM (7 of 27 patients, 26%). Hence the antral GM possesses higher susceptibility to damage of MOM under pathological conditions

Increased permeability of MOM may be associated with an impaired function of MtCK and MtAK, as is shown in cardiac cells (Kay et al., 1997a, 1997b; Saks et al., 2004, 1998). To test this association, the gastric specimens were split into two groups: one group exhibiting < 20% of Cyt c effects (here the cut-off level was set arbitrarily at 19%, a sum of mean ± SE values for 54 specimens) and the other group with > 20% effects; and I_{CK} and I_{AK} were compared for these groups. Our experiments show (Fig. 6 in Paper I) that the specimens with a larger Cyt c effect (>20%, i.e., with a larger defect in MOM) were characterized by smaller values of I_{CK} and I_{AK} than those showing less sensitivity to Cyt c (< 20%). Thus disintegration of the MOM was indeed combined with the suppressed coupling of MtCK and MtAK to OXPHOS.

The explanation for these findings may be connected to *H. pylori* infection, affecting most of the patients (71%) included in the study. It is therefore possible that coupling of MtCK and MtAK to ANT is related to the specific effects of *H. pylori* and associated inflammatory reactions exert upon mitochondrial structures. We suppose that impairment of MOM represents a common stage with adverse consequences. First, it facilitates diffusion of cytoplasmic ADP into the intermembrane compartment in which excess ADP may hinder the functional coupling between mitochondrial kinases and OXPHOS (Kay et al., 1997a; Saks et al., 2004). Second, it enables mitochondrial leak of Cyt c and MtAK and MtCK. These changes are causal for impaired intracellular energy transfer (Kay et al., 1997b; Saks et al., 1998).

In conclusion, our study demonstrates that the human corpus and antral GM possess the CK- and AK-phosphotransfer systems, which are functionally coupled to OXPHOS. We provide the first evidence that the GM inflammation, which is related to *H. pylori*, affects cellular energy metabolism by interfering with the systems of coupled reactions of AK/CK and ANT in the mitochondria. Previously several authors have shown an association of GC with suppressed expression of B-CK (He et al., 2004; Hirata et al., 1989), it appears that the whole system of intracellular energy transfer might be targeted by *H. pylori* and accompanied by inflammatory factors in the pathogenesis of different gastric diseases.

2. Oxidative phosphorylation in non-atrophic gastric mucosa: a comparative study antrum vs. corpus (Paper I)

Our study shows that mucosal oxidative capacity was higher in the corpus than in the antrum (Table 2). For the corpus GM the mean values for all respiratory rate indices (V_0, V_{Glut}, V_{Succ}, V_{COX}) were approximately twofold higher than for their antrum counterparts. These results are in accordance with the larger content of mitochondria in the corpus mucosa (Sato et al., 1978, Martin et al., 1987). The prominent oxidative capacity of the corpus GM is related to acid generation by parietal cells for which the mitochondria account for 25–45% of cell volume (Ito and Schofield, 1974; Zalewsky and Moody, 1977). To examine whether the tissue-specific differences were related not only to the amount of mitochondria but also their functional properties the ratios of respiration rates with different substrates were calculated and compared.

This analysis revealed (Table 2) that for the corpus GM, the V_{Glut}-to-V_{Succ} ratio (V_{Glut}/V_{Succ}) was higher than for the antrum GM. The V_{Glut}/V_{COX} and V_{Succ}/V_{COX} values did not differ for the two tissues. However, in the corpus GM, V_{Glut}/V_{COX} exceeded V_{Succ}/V_{COX}. Thus for the corpus GM complex I-dependent respiration was higher than complex II-dependent respiration, which implies limitation of the electron flow at the level of complex II.

In the antral GM (Table 2), in addition of smaller V_{Glut}/V_{Succ}, the value V_{Glut}/V_{COX} did not exceeded V_{Succ}/V_{COX}. Presumably, this difference in the antrum as compared with the corpus resulted in a combination of decreased complex I-dependent respiration and increased complex II-dependent respiration.

We found (Table 2) that, compared with corpus GM, antral GM exhibited lower respiration control index (RCI) with glutamate as the substrate (RCI_{Glut}) but higher RCI_{Succ}. This difference is attributable to larger deficit in V_{Glut} than in V_{Succ}, to smaller deficit in V_0 than in V_{Atr}, and to a smaller proton leak.

For the corpus GM, V_{Glut}/V_{Succ} was 1.38, which is comparable to corresponding value for other tissues, *e.g.* muscle or heart in the healthy state

(Gellerich et al., 2002; Seppet et al., 2005). The low V_{Glut}/V_{Succ} ratio (1.03) for the antrum GM (Table 2) indicates an impaired function of complex I. Moreover, a reduced V_{Glut}/V_{Succ} is considered to be a reliable index of RC deficiency at the level of the complex I, as have been observed in gene-modified animals and in human muscle disease (Gellerich et al., 2002; Rustin et al., 1991; Vahsen et al., 2004). However, the diminished activity of complex I is also seen in the mitochondria of healthy rabbit antral mucosa, as indicated by a twice lower RCI, because of reduced state 3 respiration with glutamate, compared with the corpus mitochondria (Martin et al., 1987). Thus we may suggest, that the relative deficiency of complex I is a normal property of the antral mitochondria.

Table 2. Characterization of the respiratory chain in saponin-permeabilized gastric mucosa. Values are means ± SE; the number of specimens studied is shown in parentheses. The rates of respiration (V) are given in nmol O2/min/mg wet weight. V_0 – basal respiration without ADP or ATP; V_{Glut} – ADP-stimulated respiration in the presence of glutamate and malate; RCI_{Glut} – respiration control index calculated as V_{Glut}/V_0; V_{Succ} – ADP-stimulated respiration in the presence of rotenone and succinate; V_{Atr} – respiration after inhibition of succinate-stimulated respiration by atractyloside; $RCI_{Succ} – V_{Succ}/V_{Atr}$; Proton $leak_{Norm}$ – proton leak normalized for V_{Succ}; V_{COX} – the respiratory equivalent of cytochrome oxidase activity calculated as [$V_{COX}= V_{TMPD}-V_{TMPD+NaN3}$] where V_{TMPD} and $V_{TMPD+NaN3}$ are TMPD-stimulated respiration rates before and after addition of NaN_3; V_{FCCP} – respiration in the presence of the uncoupler assessed as in Fig 3.

Parameter of Oxidative Phosphorylation	Anatomic Region of Stomach	
	Antrum	Corpus
V_0 (16)	0.164 ± 0.014***	0.300 ± 0.019
V_{Glut} (16)	0.365 ± 0.023***	0.850 ± 0.059
RCI_{Glut} (16)	2.322 ± 0.154***	2.976 ± 0.256
V_{Succ} (16)	0.378 ± 0.033***	0.650 ± 0.057
V_{Atr} (16)	0.204 ± 0.011***	0.425 ± 0.032
RCI_{Succ} (16)	1.848 ± 0.116*	1.526 ± 0.070
V_{Glut}/V_{Succ} (16)	1.027 ± 0.067**	1.376 ± 0.080
Proton $leak_{Norm}$ (16)	0.161 ± 0.032*	0.275 ± 0.044
V_{COX} (16)	0.620 ± 0.034***	1.297 ± 0.097
V_{Glut}/V_{COX} (16)	0.603 ± 0.040	0.675 ± 0.033$^{\$\$}$
V_{Succ}/V_{COX} (16)	0.615 ± 0.047	0.515 ± 0.037
V_{FCCP} (27)	0.449 ± 0.032***	0.812 ± 0.055

*$P < 0.05$, **$P < 0.01$,***$P < 0.001$ compared to corpus mucosa. $^{\$\$}P < 0.01$ compared to V_{Succ}/V_{COX} ratio in corpus mucosa.

For the first time, our study demonstrates (Fig 5) that compared to non-active gastritis, active chronic gastritis (confirmed on the basis of specific neutrophil infiltration) was associated with concerted decrease in V_0, complex I dependent ADP-stimulated respiration rate (V_{ADP}) and V_{FCCP} in the corpus GM. Besides, these parameters were augmented in the antrum GM.

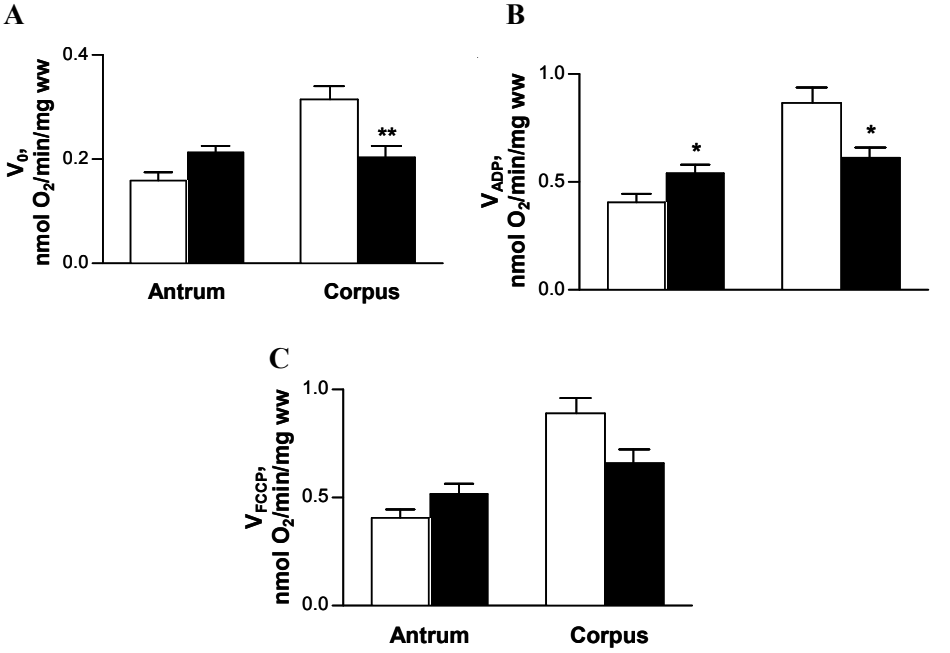

Figure 5. The effect of active chronic gastritis (filled bars, n =10 and 9 for the antrum and corpus groups, respectively) on respiratory parameters compared with absence of active process (open bars, n = 17 and 18 for the antrum and corpus groups, respectively) in permeabilized mucosa. *P < 0.05, **P < 0.01 vs. control without active process. A: V_0 – basal respiration rate. B: VADP – respiratory rate in the presence of ADP for maximal activation of OXPHOS. C: V_{FCCP} – maximum capacity of the respiratory chain estimated in the presence of uncoupler FCCP.

As most of the patients (71%) in our study were histologically *H. pylori* positive, the increased OXPHOS in the antrum GM was unexpected in the light of the evidence that *H. pylori* induces stronger oxidative stress in the antrum than in the corpus (Bayerdörffer et al., 1992; Fu et al., 1999; Ozawa et al., 2005). However, the inverse changes in OXPHOS in the antrum and corpus GM could arise from the differential effects of *H. pylori* on the balance between the antiapoptotic and apoptotic pathways in distinct parts of the stomach.

Indeed, *H. pylori* stimulates apoptosis by triggering Cyt c release (Galmiche et al., 2000; Maeda et al., 2002) and translocation of proapoptotic protein Bax (Ashktorab et al., 2004; Maeda et al., 2002) and/or the amino-terminal fragment

of bacterial cytotoxin VacA into mitochondria (Galmiche and Rassow, 2010; Galmiche et al., 2000). Based on these data and on our observation that mitochondria exhibited normal coupling of RC to ATP synthesis (no variations in RCI, data not shown), the suppressed respiration in the corpus GM suggests a decreased tissue content of mitochondria due to apoptotic loss of mitochondria and cells.

The upregulated respiration in the antrum GM observed by us may stem from the inhibition of apoptotic processes and the increased inhibition of mitochondrial biosynthesis. The *H. pylori* can also impel the cells to slow down apoptotic processes through activation of the cellular inhibitor of the apoptosis gene 2 (Yanai et al., 2003) and upregulation of cyclooxygenase-2 (COX-2) (Fu et al., 1999; McCarthy et al., 1999; Sung et al., 2000). The products of COX-2 directly inhibit nuclear factor-κB (NF-κB)-mediated apoptotic pathways via activating the peroxisome proliferator activated receptor-γ (PPARγ) (Gupta et al. 2001; Peek and Blaser 2002). After that PPARγ accelerates biosynthesis of mitochondria, thus increasing the tissue's oxidative capacity (Czubryt et al., 2003; Lehman et al., 2000). Due to the fact that *H. pylori* upregulates COX-2 in the antral GM to a greater extent than in the corpus GM (Fu et al., 1999), activation of PPARγ is expected to be more pronounced in the antral GM. This would explain the increased OXPHOS in this region observed by us.

In summary, the antral GM exhibits twice lower respiratory capacity and a relative deficiency in complex I of the RC compared with the corpus GM. These changes are probably attributable to normal tissue-specific differences. In response to active chronic inflammation OXPHOS in the corpus GM is downregulated. However, the response to the active gastritis in the antrum GM is the opposite: OXPHOS activity is increased compared with OXPHOS in non-active chronic gastritis. It could be suggest that the inverse changes in OXPHOS in the antrum and corpus GM are related to the different effects of *H. pylori* on the mitochondrial content in GM cells in distinct sections of the stomach.

3. Oxidative phosphorylation in gastric corpus atrophic mucosa (Paper II)

The results of our study (Table 3) showed that AG of the corpus GM is associated with relative changes in the activity of individual complexes. The mean values of almost all indices of respiratory rate (V_0; V_{Glut}, V_{COX} and proton leak) were about twofold lower in the atrophic GM compared whit the non-atrophic tissue. The only exception was V_{Succ}, which was not significantly decreased. Thus, our study reveals a decreased capacity of OXPHOS in the atrophied corpus GM in patients with PA.

This result is in accordance with findings that AG is associated with an abnormally low content of mitochondria in GM cells (Yin et al., 2003a, 2003b). The underlying cellular basis may include disruption of normal developmental pathways during autoimmune gastritis, resulting in depletion of parietal and

zymogenic cells, rich in mitochondria. At the same time, there occurs rapid proliferation of short-living immature gastric epithelial stem cells with blocked differentiation to end stage cells (Judd et al., 1999).

Table 3. Characterization of the respiratory chain in the gastric corpus mucosa. Values are means ± SE. The rates of respiration (V) are given in nmol O_2/min/mg wet weight. V_0 – basal respiration without ADP or ATP; V_{Glut} – ADP-stimulated respiration in the presence of glutamate and malate (indicating the activity of complex I); V_{Succ} – ADP-stimulated respiration in the presence of rotenone and succinate (indicating the activity of complex II); V_{Atr} – respiration after inhibition of succinate-stimulated respiration by atractyloside; Proton leak – difference between the rates of respiration before and after addition of antimycin A; V_{COX} – respiratory equivalent of cytochrome oxidase (complex IV) activity calculated as [V_{COX}= $V_{TMPD+Cytc}$-$V_{TMPD+Cytc+NaN3}$] where $V_{TMPD+Cytc}$ and $V_{TMPD+Cytc+NaN3}$ are TMPD+Cyt c-stimulated respiration rates before and after addition of sodium azide.

Parameter	Histological appearance		*P* value (*t* test)
	Non-atrophic (*n* = 12)	Atrophic (*n* = 12)	
V_0	0.291 ± 0.020	0.135 ± 0.011	<0.001
V_{Glut}	0.820 ± 0.060	0.366 ± 0.018[a]	<0.001
V_{Succ}	0.636 ± 0.067	0.493 ± 0.022	0.055
V_{ATR}	0.408 ± 0.041	0.202 ± 0.017	<0.001
V_{COX}	1.271 ± 0.106	0.600 ± 0.025	<0.001
Proton leak	0.173 ± 0.038	0.090 ± 0.010	<0.05

[a] $P < 0.001$ compared to V_{Succ} in atrophy group

To analyze these changes independently of individual variation, the ratios of respiration rates in the case of different substrates were calculated. The analysis shows (Fig. 6A) that in the non-atrophic corpus GM, V_{Glut}/V_{Succ} was about 1.4. Thus, in state 3, complex I-dependent respiration was higher than complex II-dependent respiration, which implies that the redox potential gained with NADH-linked substrates was higher than that gained with $FADH_2$-linked respiratory substrates. Compared to the non-atrophic GM, the atrophic GM exhibited reduced V_{Glut}/V_{Succ} but higher V_{Succ}/V_{COX} (Fig. 6B). A reduced V_{Glut}/V_{Succ} indicates that in the atrophic GM, RC deficiency is exhibited at the level of complex I (Gellerich et al., 2002; Rustin et al., 1991; Vahsen et al., 2004). Figure 6C demonstrates that in the non-atrophic GM, RCI_{Glut}, was higher compared to RCI_{Succ}, which is characteristic of mitochondria in GM with normal morphology (Martin et al., 1982).

Regarding RCI_{Glut}, mitochondria in the atrophy and non-atrophy groups were similar. Thus, low maximal respiration with complex I-dependent substrates in the AG group in comparison with the control (see Table 3) was not caused by a defect in the coupling of respiration to ATP synthesis. The data presented in

Table 3 show that although state 3 respiration with succinate was about 1.3 times lower, proton leak with the same substrate exhibited an even larger (1.9-fold) depression in the AG group compared with the non-atrophy group, resulting higher RCI_{Succ} than that noted in the non-atrophic GM (Fig 6C). Thus, our study demonstrates that mitochondria in the atrophic GM have a markedly tighter coupling between respiration and phosphorylation, which enabled them to maintain V_{Succ} close to that observed in the non-atrophic GM.

In general, it appears that AG of the corpus GM is associated with a metabolic shift characterized by a lower tissue content of mitochondria and alterations in the individual properties of mitochondria in a mode that weakens their ability to use NADH-linked substrates but favors use of the $FADH_2$-linked substrate, succinate, to fuel OXPHOS.

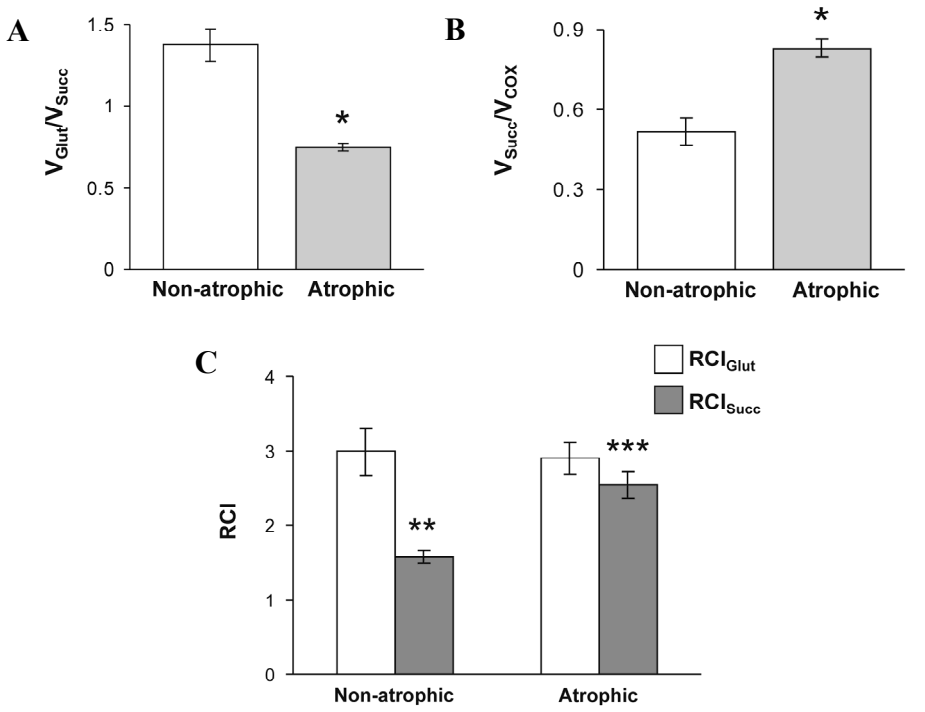

Figure 6. Changes in the ratios of the respiration rates of the respiratory chain complexes in the atrophic corpus mucosa. A. The V_{Glut}/V_{Succ}, ratio of ADP-stimulated respiration rate in the presence of glutamate and malate (indicating the activity of complex I) to ADP-stimulated respiration rate in the presence of rotenone and succinate (indicating the activity of complex II). B. The V_{Succ}/V_{COX}, ratio of the V_{Succ} to V_{COX} (complex IV)-dependent respiration rate. C. RCI, respiratory control index; RCI_{Glut}, the ratio of V_{Glut} to basal respiration (V_0) without ADP or ATP; RCI_{Succ}, ratio of V_{Succ} to respiration rate after inhibition of succinate-stimulated respiration by atractyloside; n = 12 in both groups. *$P < 0.001$ compared to the non-atrophy group; **$P < 0.001$ compared to RCI_{Glut}, ***$P < 0.001$ compared to RCI_{Succ} in the non-atrophy group.

It is well known that serum pepsinogen levels are important biochemical markers for monitoring peptic secretion and the status of the GM in healthy subjects and in patients with gastrointestinal diseases (Agréus et al., 2012; di Mario and Cavallaro, 2008; Gritti et al., 2000). Our study shows (Fig. 4) that the serum S-PGI levels of the studied patients (the control and the PA groups) correlated fairly well with V_{Glut}/V_{Succ}. These levels correlated also with V_{Glut} ($r = 0.684$, $P = 0.0005$, $n = 22$), but not with V_{Succ} (not shown). These findings suggest that the low secretory activity of the gastric oxyntic mucosa can be related to complex I deficiency developing in association with atrophy of the corpus mucosa.

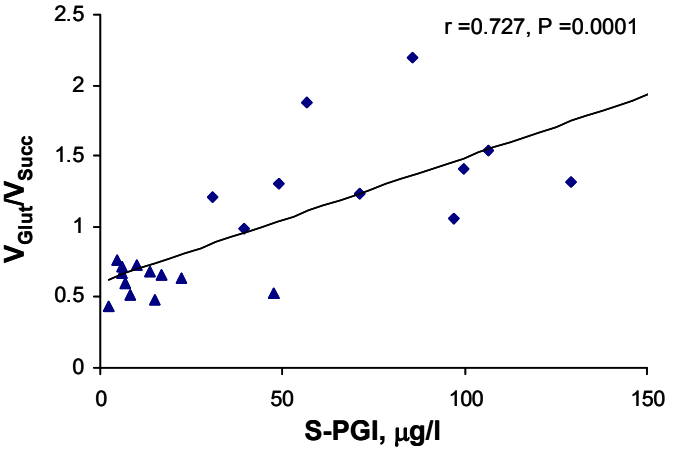

Figure 7. Correlation between relative deficiency of respiratory chain complex I and serum pepsinogen I (S-PGI) level. Patients with non-atrophic gastritis (squares) and patients with atrophic gastritis (triangles); V_{Glut}/V_{Succ}, the ratio of ADP-stimulated respiration rate in the presence of glutamate and malate (indicating the activity of complex I) to ADP-stimulated respiration rate in the presence of rotenone and succinate (indicating the activity of complex II). $n = 22$.

In conclusion, GM atrophy in PA patients is associated with remodelling of the system of OXPHOS, which may limit the secretory capacity of mucosal cells. Analysis of mitochondrial function *in vivo* in gastrobiopsy specimens may offer a novel diagnostic means to identify defects in the function of the mitochondrial RC as early precancerous changes in a diseased GM.

4. Oxidative phosphorylation in gastric cancer cells (Paper III)

Our study demonstrates (Table 4) that in the cancer group and in the adjacent GM group of patients with GC, the mean values of respiratory rates, corresponding to the activity of different RC complexes (V_{Glut}, V_{Succ}, and V_{COX}), were twofold lower than in the control group. This finding implies that development of GC is associated with strongly reduced activity of OXPHOS not only in cancerous GM but also in the nonmalignant GM adjacent to the GC, as compared with normal OXPHOS activity. Since AG was found in the adjacent mucosa of almost all patients with GC and a similar decrease in respiratory rate was found in he atrophic corpus GM of patients with PA (Paper II), it is possible that decreased respiratory capacity in the group of adjacent GM stems from the loss of mitochondria-rich parietal cells due to mucosal atrophy preceding the development of the cancerous GM.

A major new observation of our study was that both the cancerous and adjacent GM exhibited a relative deficiency of the complex I of the mitochondrial RC. Fig. 5 demonstrates that the V_{Glut}/V_{Succ} ratios were significantly reduced in both cancer patient groups. Given the similar respiratory control by ADP in the presence of glutamate/malate (RCI_{Glut}) in all groups (Table 4), this defect was not caused by the impaired coupling of respiration to ATP synthesis. In order to relate these results to the cancer cell phenotype, the alterations observed in GC patients were compared to the indices of mitochondrial respiratory function in human GC cell lines MKN28 and MKN45. The complex I deficiency has been earlier observed in the human ovarian cancer tissue (Lim et al., 2011), in renal oncocytoma (Simonnet et al., 2003), in thyroid oncocytoma (Bonora et al., 2006) and in the corpus GM of patients suffering from AG (Paper II) as well as in conditions of chemically induced carcinogenesis in the rat liver (Boitier et al., 1995) the reduced V_{Glut}/V_{Succ} ratio may expected in GC cell lines as well.

Our study demonstrates also (Fig. 8) that the GC cell lines MKN28 and MKN45 exhibited even stronger suppression on the V_{Glut}/V_{Succ} ratio than that detected in the cancer GM and the adjacent GM groups. Thus, the impaired function of complex I of the RC is an inherent property of the GC cell line, at least in conditions of oxidation of glutamate and malate. The observation concerning this change became evident in the cells adjacent to cancer mucosa suggests that impairment of complex I may be an early sign of phenotypic shift from a normal to a cancerous tissue.

Table 4. Characterization of respiratory parameters in the gastric corpus mucosa of patients without gastric cancer (Control) and patients with gastric adenocarcinoma, in specimens obtained from the tumour tissue (Cancer) and from the mucosa adjacent to the cancer (Adjacent). The means ± SE of parameter values are given for the experiments performed according to the protocol shown in Fig. 1. The rates of respiration (V) are expressed in nmol O_2/min/mg protein. V_0 – basal respiration without ADP or ATP; V_{Glut} – ADP-stimulated respiration in the presence of glutamate and malate (indicating the function of the respiratory chain complex I); RCI_{Glut} – respiration control index calculated as a ratio of maximal ADP-dependent respiration rate in the presence of glutamate and malate to basal respiration rate without ADP, V_0; V_{Succ} – ADP stimulated respiration in the presence of rotenone and succinate (characterizing the function of complex II); V_{Atr} – respiration after inhibition of succinate-stimulated respiration by atractyloside; RCI_{Succ} – ratio of ADP-stimulated respiration in the presence of succinate when respiratory chain complex I is inhibited by rotenone, to respiration rate after inhibition of succinate-stimulated respiration by atractyloside; Proton leak – measured indirectly as state 4 respiration equal to the difference between the respiration rates with atractyloside and antimycin A; V_{COX} – the respiratory equivalent of cytochrome oxidase (complex IV) activity calculated as $[V_{COX}=V_{TMPD}-V_{TMPD}+ NaN_3]$ where V_{TMPD} and $V_{TMPD+NaN3}$ are TMPD-stimulated respiration rates before and after addition of NaN_3.

Parameter	Control (n = 10)	Adjacent (n = 6)	Cancer (n = 6)
V_0	0.29 ± 0.03	0.14 ± 0.03**	0.13 ± 0.01***
V_{Glut}	0.89 ± 0.03	0.39 ± 0.06***	0.34 ± 0.04***
RCI_{Glut}	3.21 ± 0.30#	3.21 ± 0.19	2.67 ± 0.24
V_{Succ}	0.71 ± 0.08	0.49 ± 0.05	0.40 ± 0.05*
V_{ATR}	0.48 ± 0.05	0.19 ± 0.04***	0.18 ± 0.01***
RCI_{Succ}	1.48 ± 0.06	2.80 ± 0.31***	2.17 ± 0.24*
Proton leak	0.23 ± 0.04	0.13 ± 0.02	0.10 ± 0.02*
V_{COX}	1.31 ± 0.13	0.74 ± 0.08**	0.61 ± 0.08**

*P < 0.001, **P < 0.01, *P < 0.05, compared to mucosa of the control group. # P < 0.001 compared to the RCI_{Succ} of the control group.

Table 4 demonstrates that in the non-atrophic corpus GM, RCI_{Glut} almost doubled RCI_{Succ}. It means, that the succinate-dependent OXPHOS was better preserved than complex I-dependent process in the GM of patients with GC. This difference stems from relatively higher V_{Atr} in comparison with glutamate/malate dependent V_0. The high value of V_{Atr} can be explained by the higher mitochondrial membrane potential during succinate oxidation, which promotes proton leak (Hawkins et al., 2010; Lim et al., 2011). Our study shows (Table 4) that the development of GC was accompanied with suppression on V_{Glut} to a relatively larger extent (by 2.7 times) than in V_{Succ} (1.8 times) as compared with the control parameters. In addition, the succinate dependent proton

leak (V_{ATR}) was diminished. These changes combined to result in increased RCI_{Succ} compared to the corresponding parameter in the control group. This suggests that in GC cells the energetic limitations due to downregulation of OXPHOS and insufficient RC complex I can be overcome by shifting the preferable substrate for oxidation from pyruvate to succinate.

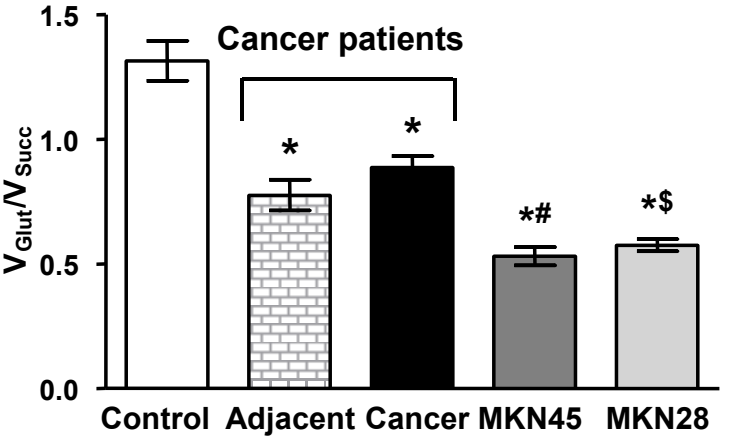

Figure 8. Deficiency of the respiratory chain complex I of the mitochondria in the endobiopsy samples from patients with gastric cancer and in gastric cancer cell lines. The V_{Glut}/V_{Succ} – ratio of ADP-stimulated respiration rate in the presence of glutamate and malate (indicating the activity of complex I) to ADP-stimulated respiration rate in the presence of rotenone and succinate (indicating the activity of complex II). Groups: Control (n = 10) – normal corpus mucosa; Cancer (n = 6) – tissue of gastric cancer; Adjacent (n = 6) – non-cancerous mucosa adjacent to cancer tissue; MKN45 (n = 8) – cell line derived from poorly differentiated gastric adenocarcinoma; MKN28 (n = 8) – cell line originating from moderately differentiated gastric adenocarcinoma. *P < 0.001 compared to the control group; #P < 0.01 compared to cancer; \$$P$ < 0.05 compared to cancer.

In conclusion, the relative deficiency of the RC complex I but improved adenylate control over succinate-dependent respiration, registered by us, may be an intrinsic property of the GC and his precursor cells Therefore, quantitative assessment of these phenomena by oxygraphy of endobiopsy samples may be of diagnostic importance to detect early changes in GM leading to GC. How this remodeling of the OXPHOS system, detected by us, influences the development and life of the GC, needs further investigations.

5. Development of gastric cancer: possible role of altered mitochondrial function (Papers II and III)

To address the role of mitochondria in gastric carcinogenesis, we characterized the function of OXPHOS in the biopsies of GM taken from the corpus region of patients suffering from chronic inflammation, AG (PA patients), and intestinal type GC.

A major new finding of our study was that mitochondria in atrophic corpus GM, in GC tissue and in GC cell lines MKN45 and MKN28 exhibited relative deficiency of RC complex I and improved respiratory control by ADP in the presence of succinate as compared to non-atrophic corpus GM (Papers II and III). The OXPHOS in the atrophied corpus GM (Table 3 and 4) was also downregulated as compared to the control group. This suggests that in intact cells, energetic limitations due to downregulation of OXPHOS and insufficient complex I can be overcome by shifting the preferable substrate for oxidation from pyruvate to succinate. Is known that the RC complex II has been considered as pH sensor for apoptosis and several of its components appear to serve as tumor suppressors, and, vice versa, mutations of the components of complex II are associated with carcinogenesis (Lemarie and Grimm, 2011). It is remarkable that Ras-dependent transformation leads to up-regulation of the activity and components of SDH (Baracca et al., 2010; Gough et al., 2009; Hu et al., 2012). Thus, alterations in the balance between the functions of complex I and complex II of the mitochondrial RC in AG and GC cells, found by us, may lead to important pathophysiological and clinical consequences. At present, it is unclear whether and how the relative complex I deficiency observed in our study, may be related to transition of GM cells from proliferation to carcinogenesis.

First, the role of mitochondrial ROS deserves attention. It has been proposed that the RC reacts as a cellular O_2 sensor, by releasing ROS due to the retarded flow of electrons along the RC, which reduces the cytochromes and increases the lifetime of the ubisemiquinone radical in complex III (Guzy and Schumacker 2006). It is known that most of mitochondrial ROS is produced at the level of complex I, where unpaired electrons are transferred to O_2 to generate superoxide, and suppression of the complex I significantly compels ROS production. (Barrientos and Moraes, 1999). Thus, the relative inhibition of complex I observed in the atrophic GM should most likely be accompanied with ROS production. This eventually culminates not only with inflammation and cell proliferation, the typical features of gastritis, but also with carcinogenesis. Indeed, among a variety of the mechanisms underlying cancer development, MtDNA mutations induced by ROS represent one of the key elements; and because respiratory complexes I, III and IV contain subunits encoded by MtDNA (Brandon et al., 2006; Kelly and Scarpulla, 2004), deterioration of complex I may be a major outcome. Mutations in a number of subunits of complex I can be accounted for by its impaired function. These mutations give rise to exces-

sive ROS production, which secondarily affects other complexes of RC (Barrientos and Moraes, 1999; Brandon et al., 2006; Lemarie and Grimm, 2011).

Mitochondrial ROS can also activate the hypoxia-inducible transcription factor 1α (HIF-1α) (Brunelle et al., 2005; Chandel et al., 2000; Emerling et al., 2005; Guzy and Schumacker, 2006; Iyer et al., 1998; Mansfield et al., 2005; Sanjuán-Pla et al., 2005; Semenza, 2002). HIF-1α is a potent inducer of gene transcription all genes encoding glycolytic enzymes, glucose transporters, vascular endothelial growth factor, erythropoietin, and insulin-like growth factor (Gerald et al., 2004; Iyer et al., 1998), which enables the to survive during the hypoxia period. Interestingly, in the early stages of carcinogenesis, even before the histological evidence of angiogenesis or invasion, tumor cells in different tissues exhibit overexpression of HIF-1α (Zhong et al., 1999). In line with these data, analysis of the gastric biopsies of the normal GM, *H pylori*-associated gastritis, intestinal metaplasia, dysplasia, and intestinal and diffuse cells adenocarcinoma revealed progressively increasing expression of HIF-1α and HIF-2α along with this gastric carcinogenic sequence (Griffiths et al., 2008, 2007). Moreover, data concerning altered succinate metabolism in the mitochondria of the atrophic GM may also regulate transition of mucosal cells into a tumor state, via upregulating the HIF-1α-mediated pathway. Agani and colleagues (Agani et al., 2000) have shown that the activity of HIF-1α depends directly on the status of the mitochondrial RC, or, more specifically, on the balance between complex I-dependent and complex II-dependent respiration. By using a cybrid cell model of the altered activity of complex I *in vivo*, these authors found that partial complex I deficiency effectively prevented the hypoxic induction of HIF-1α protein, while succinate restored the hypoxic response in cybrid cells (Agani et al. 2000).

Second, a defect in complex I may be regarded as a common and specific marker of adaptation of the mitochondria in response to stressors causing proliferation of immature epithelial cells as seen in murine autoimmune gastritis (Judd et al., 1999) and in human *H. pylori*-associated gastric intestinal metaplasia (Leung et al., 2001; Miwa et al., 1996). In these processes, rearrangements of the activity of mitochondrial enzymes and in the RC may be similar to the corresponding processes occurring in rapidly growing cancer and yeast cells. For example, HeLa cells possess mitochondria with a truncated Krebs cycle and probably a defect in complex I, as a consequence of which they are unable to utilize NADH-linked substrates and switch to succinate utilization (Piva and McEvoy-Bowe, 1998). An analogous mitochondrial phenotype has been observed in chronologically aging yeast cells. Namely, the shunting of the complete Krebs cycle by the glyoxylate cycle with concomitantly increased rates of succinate formation and oxidation may underlie their higher viability (Samokhvalov et al., 2004). Our study demonstrates a novel outcome of these changes – improved coupling between oxidation and phosphorylation with succinate.

Third, the enhanced activity of complex II in GC cells may support manifestation of the cancerous phenotype of the cell by increasing its resistance to hypoxia through preventing loss of the membrane potential in hypoxic cells (Hawkins et al., 2010). It has been proposed that while normally complex II operates as a succinate-UQ reductase, in hypoxic conditions it operates in a reverse direction, i.e. as a NADH-fumarate reductase system (Tomitsuka et al., 2010). This mechanism enables generation of the mitochondrial membrane potential and thus drives ATP synthesis in the absence of oxygen, thereby supporting the survival of cancer cells in conditions when the oxygen availability is limited (Tomitsuka et al., 2010). The observation concerning vitamin E analogues, such as a-tocopherol succinate, which inhibit SDH, can induce apoptosis selectively in cancer cells, by acting mainly through rapid generation of ROS (reviewed by Ralph et al., 2010). This supports the suggestion that the GC cell's life strongly relies upon the function of complex II.

Another interesting finding in our study was the fact that the extent of deficiency of RC complex I correlated with S-PGI level (Fig. 7). In clinical practice, it is quite important whether the S-PGI and S-PGII screening system could be used for detection of GC, especially at early stage. In case of intestinal GC, the PG screening system is useful and works well because S-PGI is an excellent biomarker of corpus AG (the precancerous state of intestinal GC). It can be assayed from serum/plasma, and its levels decrease with increasing grade of AG (Vauhkonen et al., 2006; Agréus et al., 2012). In diffuse GC the Correa`s cascade is not the prevailing sequence in morphogenesis. Non-atrophic *H. pylori* gastritis is the most common underlying or co-existing gastric condition in patients with diffuse type GC (Vauhkonen et al., 2006). Recent studies showed that the risk of diffuse-type GC increased as level of S-PGII increased (Yanaoka et al., 2008; Ito et al., 2012). We found that in case of non-atrophic active *H. pylori* gastritis the function of the OXPHOS system in the corpus and antrum GM was significantly affected (Fig. 5). Therefore in the future it could be interesting to apply our oxygraphic method for studying the OXPHOS system (especially the ratios of the respiratory activity of the RC complexes) in the GM of patients suffering from non-atrophic gastritis and diffuse GC, together with serum screening for anti-*H. pylori* antibody, S-PGI and particularly S-PGII. Such kind of research may help find better serum screening criteria for identifying subjects at high risk for diffuse type GC.

In summary, it appears that suppressed OXPHOS and the deficit of complex I in the RC may be important factor supporting transition from chronic gastritis to GC. Oxygraphic measurement o activity of complex I to activity of complex II activity ratios may therefore serve as a valuable diagnostic tool for detecting the metabolic shift to carcinogenesis in GM cells. The prognostic value requires further experimental and clinical evaluation.

CONCLUSIONS

The conclusions of the study are the following:

1. In the human gastric corpus and antral mucosa there exist the AK- and CK-phosphotransfer systems, which are functionally coupled to OXPHOS.
2. The respiratory capacity of the OXPHOS in non-atrophic cases is approximately two times higher in the corpus mucosa compared to the antral mucosa. In response to active chronic inflammation OXPHOS in the corpus mucosal cells is downregulated. However, the response to the same disease in the antrum mucosal cells is the opposite: respiratory capacity is increased compared with respiratory capacity in non-active chronic gastritis.
3. The mitochondria of the non-atrophic mucosal cells of the antrum display a relative deficiency of respiratory chain complex I as compared to the corpus mucosal cells. Also the mitochondria of the antral mucosal cells show higher susceptibility to outer membrane damage compared with the mitochondria of the corpus mucosal cells.
4. The gastric corpus mucosa of patients with corpus dominant atrophic gastritis exhibits decreased OXPHOS capacity with a relative deficiency of respiratory chain complex I as compared to the non-atrophic gastric corpus mucosa. These changes correlate with decreased secretion of S-PGI.
5. Gastric corpus cancer is associated with decreased OXPHOS capacity and a relative deficiency of the respiratory complex I, but with improved coupling of succinate oxidation (function of complex II) to ATP generation in the mitochondria of the tissues of gastric adenocarcinoma and of the adjacent atrophic gastric corpus mucosa as well as in gastric cancer cell lines MKN28 and MKN45, compared to the non-atrophic gastric corpus mucosa.

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SUMMARY IN ESTONIAN

Oksüdatiivne fosforüülimine erinevate mao limaskesta haiguste korral

Kuna diagnoositud maovähk (MV) on tihti jõudnud hilisesse staadiumisse ja selle prognoos on tavaliselt halb (viie aasta elulemus 25%), on kõik seisukohad, mis puudutavad MV tekkimist ja arengut, ebaselged. Seetõttu on eriti oluline vähieelsete seisundite iseloomustamine ja MV varajase diagnoosimise meetodite otsimine.

Maovähi bioloogilist arengut iseloomustab nn Correa kaskaad, mis väljendub järgnevuses: põletik→atroofia→düsplaasia→vähk ja baseerub kolmel üksteisega läbipõimunud protsessil. Esimene on krooniline aktiivne põletik, mis loob aluse geno- ja fenotüübilistele muutustele maolimaskestas (ML). Teine on apoptoosi ja rakkude jagunemise tasakaalu häirumine, mis väljendub ML õhenemisena atroofilise gastriidi (AG) korral või rakkude vohamisena. Kolmas on rakkude diferentseerumise häirumine, mille käigus toimub maonäärmete asendumine lihtsamat tüüpi näärmetega (intestinaalne metaplaasia). Kõigi kolme protsessi mõjutamisel on leidnud rohkesti kinnitust bakter *Helicobacter pylori* osalemine. Lisaks, lubavad olemasolevad andmed oletada, et eelpoolmainitud protsesside aluseks on ka spetsiifilised muutused rakkude energeetilises ainevahetuses.

On kirjeldatud, et vähirakkudes on mitokondrites toimuv oksüdatiivne fosforüülimine ja selle hingamisahela funktsioneerimine häiritud. Üheks hingamisahela toimimise muutuste tagajärjeks on hapniku vabade radikaalide teke. Hapniku vabadel radikaalidel on aga oluline roll põletikuprotsessides, rakukahjustuste tekkes ja apoptoosi algatamisel. Seetõttu on meie töö ülesanne uurida oksüdatiivse fosforüülimise ja hingamisahela komplekside funktsiooni ML rakkudes nii kroonilise põletiku, AG kui ka MV korral (järgides Correa kaskaadi) ja leida häired, mis viitaksid varastele vähieelsetele muutustele.

On näidatud, et mitmetes rakutüüpides (lihased, spermatosoidid, närvitakud) töötavad energia (energiarikka fosfaatgrupi) transpordi süsteemid, mis koosnevad adenülaatkinaasi (AK) ja kreatiinkinaasi (CK) rakusisestest- ja mitokondriaalsetest isovormidest. Nende süsteemide ülesandeks on tagada adekvaatne tagasiside ATP kasutamise ja tootmise vahel. Meile teadaolevalt pole energiatranspordi süsteemide toimimist ML rakkudes terviklikult uuritud ja see on meie töö teine oluline ülesanne

Uuringutes osalesid järjestikused patsiendid, kes saadeti diagnostiliseks gastroendosskoopiaks TÜ Kliinikumi. Kolmes uuringus osalesid: 60 patsienti mitte-atroofilise kroonilise gastriidiga, 12 pernitsioose aneemia patsienti (lõppjärgus AG) ja 6 MV patsienti. Lisaks uuriti ka MV rakuliine. Töös kasutati peamise meetodina ML gastrobiopsiates koe rakumembraanide läbilaskvuse suurendamist (permeabiliseerimist) saponiiniga. Permeabiliseeritud koepreparaatides määrati hapnikutarbimist. Hingamisahela komplekside hingamisaktiiv-

suse ja energiatranspordi süsteemide toimimise uurimiseks kasutati erinevaid substraat-inhibiitor tehnikaid.

Doktoriväitekirja eesmärgid olid:

1. Iseloomustada AK- ja CK energiatranspordi süsteeme inimese maolimaskestas.
2. Hinnata oksüdatiivse fosforüülimise respiratoorset suutlikkust antrumi ja korpuse mitte-atroofilises maolimaskestas aktiivse ja mitte-aktiivse kroonilise gastriidiga patsientidel.
3. Iseloomustada kvalitatiivseid erinevusi mitokondrite hingamises mao antrumi ja korpuse limaskestas mitte-atroofilise gastriidi korral.
4. Iseloomustada võrdlevalt oksüdatiivse fosforüülimise funktsiooni korpusdominantse gastriidiga pernitsioosse aneemia patsientide maokorpuse limaskestas ja mitte-atroofilise korpuse maolimaskestaga patsientidel, seoses S-PGI tasemega.
5. Iseloomustada võrdlevalt oksüdatiivse fosforüülimise funktsiooni inimese maokorpuse limaskestas maovähi patsientidel, korpuse mitte-atroofilise limasksestaga patsientidel ning inimese maovähi rakuliinides MKN28 ja MKN45.

Töö tulemustest järeldub:

1. Inimese mao korpuse ja antrumi limaskestas esinevad AK- ja CK energiatranspordi süsteemid, mis on funktsionaalselt seotud oksüdatiivse fosforüülimisega.
2. Maokorpuse mitte-atroofilises limaskestas on oksüdatiivse fosforüülimise respiratiivne suutlikus ligikaudu kaks korda suurem kui mao antrumi limaskestas. Vastusena krooniliselе aktiivsele põletikule oksüdatiivne fosforüülimine korpuse maolimaskestas väheneb. Kuid antrumi maolimaskestas on vastus samale haigusele vastupidine, seal oksüdatiivne fosforüülimine suureneb, võrreldes mitte-aktiivse kroonilise põletikuga.
3. Antrumi mitte-atroofilise maolimaskesta mitokondrites esineb hingamisahela kompleks I suhteline defitsiit, mida korpuse limaskestarakkudes ei täheldatud. Samuti esineb antrumi limaskestarakkude mitokondrites suurem vastuvõtlikkus mitokondri välismembraani kahjustustele kui korpuse limaskesta rakkude mitokondrites.
4. Võrreldes mitte-atroofilise korpuse limaskestaga avaldub maokorpus dominantse atroofilise gastriidiga patsiesntide korpuse maolimaskestas vähenenud oksüdatiivse fosforüülimise suutlikkus koos hingamisahela kompleks I suhtelise defitsiidiga. Need muutused korreleeruvad langenud S-PGI sekretsiooniga.
5. Võrreldes korpuse mitte-atroofilise limaskestaga on maokorpuse vähk seotud vähenenud oksüdatiivse fosforüülimise suutlikkusega ja hingamisahela kompleks I defitsiidiga, kuid seotus suktsinaadi oksüdatsiooni (kompleks II funktsioon) ja ATP tootmise vahel on paranenud, nii mao adenokartsinoomis

kui ka seda ümbritsevas maokorpuse atroofilises limaskestas ning maovähi rakuliinides MKN28 ja MKN45.

Seega toimuvad atroofiliseses korpuse MLs ja MV rakkudes oksüdatiivse fosforüülimise süsteemi spetsiifilised muutused, mis väljenduvad hingamisahela kompleks I suhtelise defitsiidina ning kompleks II funktsiooni paranemisena. Kuna kompleks I suhteline defitsiit ilmneb juba vähieelse seisundi (AG) korral ja süveneb MV rakkudes, võib sellel olla oluline roll MV arengus.

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