



**Effects of Proton Pump Inhibitors on
the bioactivation of dietary nitrate
during submaximal exercise**

Master thesis

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Abstract

Aim: The purpose of the study is to show the influence of Proton Pump Inhibitors (PPI in form of esomeprazole) on the bioactivation of dietary nitrate (sodium-nitrate solution) in submaximal exercise, through affecting the gastric pH.

Method: Randomized, doubled-blinded, placebo-controlled and crossover study with six subjects (mean \pm SD, age 29 ± 5 years, height 170 ± 5 centimeters, weight 70 ± 5 Kg, BMI $24,36 \pm 1,75$ Kg/m² blood pressure $119/ 77 \pm 6$ mmHg, 3 male and 3 female). They were tested in two different trials. Every trial consisted of two parts. One part was cycling on 4 different submaximal stages (80W/60RPM, 80W/90RPM, 120W/60RPM, 120W/90RPM) for 5 minutes each, with 90 minutes rest in between. The same protocol was repeated. In the beginning of the resting time a sodium nitrate solution (NaNO_3^- , 10mg/kg body weight) was ingested. VO_2 , VCO_2 , RER, VE, Lactate, Glucose, heart rate and blood pressure were recorded. Venous blood samples were taken. Whether esomeprazole (10mg) or a placebo were taken 24h, 12h and directly before being tested in both trials. Subjects were pleased to have a nitrate poor diet starting when taking the pills. An information sheet was provided.

Results: No significant differences were found between the post values and the treatment. Tendencies of a higher oxygen consumption when taking esomeprazole (2,62%) in comparison to placebo (0,11%) were observed. Systolic BP decreased by 3,91% with the placebo while it decreased just 2,04% with esomeprazole after intake. Sex-specific differences occurred in the metabolism of esomeprazole and dietary nitrate. RER showed a significant post nitrate difference between the female and male participants with $t=.006$ and a significance in pre-dietary nitrate intake. VE in female ($40,79 \pm 7,20$ L/min) and ($50,03 \pm 10,09$ L/min) in male were as well significant ($t=.017$).

Conclusion: Tendencies of effects of PPI are seen in the post-values of VO_2 and BP after intake of dietary nitrate. Gender-differences are shown in RER and VE. More research is needed to see the impact of dietary nitrate on the human body under submaximal load.

Keywords: nitrate-nitrite-nitric oxide pathway, proton pump inhibitors, gender-specific metabolism, NaNO_3^- supplementation, submaximal load, blood pressure, VO_2

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1. Introduction

1.1. Background

What we eat is one of the most important aspects of our health. To use the ingested macronutrients (carbohydrates, proteins and fats) we have to digest them into their smallest components. Without this digesting procedure, food would be useless for the body. There are as well some micronutrients that need to be bioactivated to be used by the body.

“Bioactivation is the process where enzymes or other biologically active molecules acquire the ability to perform their biological function, such as inactive proenzymes being converted into active enzymes that are able to catalyze their substrates into products to produce (by definition) metabolites that are more toxic than their parent compounds.”

(P. Gupta, 2018, p. 375)

One of those micronutrients is nitrate. Nitrate is an inorganic anion that is mostly found in green leafy vegetables in our daily nutrition and has a natural appearance (Clements et al., 2014). 60-80% of dietary nitrate is consumed through our daily diet (Lundberg et al., 2004). The main sources of nitrate are lettuce, beetroot and spinach (Lundberg, Weitzberg, & Gladwin, 2008). Other sources are cigarette smoke, car exhaust fumes and drinking water (Norman & Keith, 1965).

The bioactivation is guaranteed by different pathways that are used to convert ingested dietary nitrate to bioactive nitric oxide (NO) that can be used by the body (Larsen et al., 2010). It is important to differentiate between ingested dietary nitrate and nitrite (NO₂⁻). Nitrite is used as a salt for food preservation whereas potassium nitrate (NO₃, saltpeter) is used to maintain color and flavor in processed and cured meats (Clements et al., 2014). The differentiation between those different forms leads to different effects on the health system.

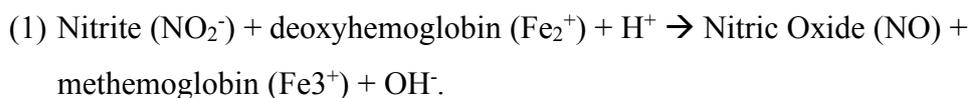
Yet it is not sure how drugs as PPI effect the bioactivation of nitrate. In this thesis I tested the difference after taking a NaNO₃⁻ solution after a (1) 24 hour loading time of esomeprazole and (2) compared to a 24-hour loading time of a placebo in VO₂, VCO₂, RER, HR, BP, lactate and glucose. The values are measured through indirect measurements by knowing that PPI's increase the gastric pH, equivalent to a more alkaline level and less bioactivation of NO under those conditions are expected.

1.1. Vasodilatation

Nitric oxide is suggested to have several positive physiological functions (Larsen et al., 2010). One of the most well-known positive effects of nitrate is the reduction of blood pressure through vasodilatation. NO is one of the most well-known vasodilators (BP goes down) (Lundberg, Weitzberg & Gladwin, 2008). The role of maintaining the basal tone through the slow and steady release of NO into blood vessels is huge (Vallance et al., 1989). Leading to vasodilatation and the regulatory role of NO to regulate the blood flow to the muscles (Larsen et al., 2007).

Vasodilatation is associated with a lowering of blood pressure and is mediated by cyclic guanosine monophosphate (c-GMP) and the relaxing impact by opening Ca_2^+ channels at the cell membrane (Bondonno et al., 2016; Heckmann & Dudel, 2010).

The dependence of the whole process is determined by NO, hemoglobin (Hb) and the micronutrient iron (Fe). Lundberg et al. (2008) described the process which leads to vasodilatation by two different chemical reactions.



This first part of the reaction is similar to the bacterial reaction which occurs in the mouth when ingesting dietary nitrate (Lundberg et al., 2008).



The reactions describe the idea that hemoglobin influences the process of vasodilatation by acting as an allosterically regulated nitrate reductase (Lundberg et al., 2008). Hemoglobin-mass correlates with performance and defines the level of Fe_2^+ which influences the whole reaction (Prommer et al.; 2008). Deoxyhemoglobin (Fe_2^+) is reversible by connecting to and from oxygen (Jelkmann, 2010). Influenced by the pH and oxygen concentration, Fe_2^+ changes the chemical affinity (Cosby et al., 2003). Arising thereby that NO secretion is dependent by the reaction with deoxyhemoglobin (Cosby et al., 2003). Deoxyhemoglobin reduces NO_2^- by a rate 30 times faster than normal hemoglobin (Huang et al., 2005; Shiva et al., 2007). As hemoglobin is deoxygenated under exercise, the reaction enhances the production of NO out of the reaction with deoxyhemoglobin (Rassaf et al., 2007; Shiva et al., 2007). This is

confirmed by Larsen et al. (2007), which showed that nitrate supplementing lowers the oxygen consumption. Another factor is the influence of the pH during submaximal load. As more oxygen is used, more carbon dioxide (CO₂) is produced. The Bohr-effect shifts the Hb binding-affinity while increasing CO₂-production and the associated acidosis (Jelkmann, 2010). These conditions lead to a lower bindings-affinity of Hb which results in more usable O₂ in the tissues. The higher the metabolic activity of the tissues the easier the release of O₂ by Hb (Jelkmann, 2010). This allosteric reduction from NO₂⁻ to NO by Fe₂⁺ peaks with a 50% oxygen saturation, while the submaximal performance aerobic energy supply is maintained (Lundberg et al., 2008).

At the same time, methemoglobin (met-Hb) is released. Met -Hb is suggested with adverse characteristics that can lead to poisoning (Lundberg et al., 2008). Knowing met- Hb (Fe₃⁺) is not able to connect to oxygen leads to a much higher oxygen affinity of Fe₂⁺ (Jelkmann, 2010).

In addition, vasodilatation can also be influenced by different physiological circumstances. The main aspect of vasodilatation is to reduce the pressure in the vessels. BP is associated with an indirect better perfusion of the blood vessels and defined as the pressure existing while the heart is beating and circulating blood through the pulmonary and systemic circuits in the vessel walls (Lundberg et al., 2008).

The cardiac cycle is divided into two phases. The first phase is the diastolic phase in which the heart- ventricles are relaxed and filled with blood. The second phase is the systolic phase where the ventricles pump the blood into the arteries. These cardiac cycle phases are affected by the cardiac output at the systolic phase and the peripheral vascular resistance.

Preload, afterload, and contractility determine the cardiac output while using the Frank-Starling law (Glomer et al., 1985). Preload is defined as the myocardial tension which stretches the ventricle prior to the diastole (Vincent, 2008). The Frank-Starling law asserts that the greater the cells are stretched in the heart the greater the force of contraction is (Vincent, 2008). Overall, a bigger blood volume leads to a greater stretch. The blood pumped back from the body to the heart will determine the ventricular filling which leads to a more powerful contraction and a greater cardiac output.

Furthermore, ejection fraction increases and the volume for the left ventricle becomes greater. Afterload is the pressure the heart has to overcome to eject blood against the existing arterial blood pressure (Vincent, 2008). Contractility is how much power the heart can produce by itself depending on the inotrope hormones (Vincent, 2008). By lowering the BP, the risk of a stroke and myocardial infarction decreases through better nourishment of the vessels

(Lundberg et al., 2008). Saying this implies less cardiac stress and a more sufficient and energy efficient work phases of the heart.

Additional positive effects are investigated by Clements et al. (2014) in anti-cancer and anti-inflammatory properties. Taking away those positive properties of NO can lead to serious health issues. The chronic use of PPI is suggested with a higher risk of cardiovascular diseases (Bhatt et al., 2010). Over the last decade an overutilization has been described by Bashford, Norwood, & Chapman (1998). They claim that PPI prescriptions have risen 10-fold in a 4-year period and repeated descriptions reached 71% in total.

1.2. Proton Pump Inhibitors (PPI)

The most potent drug used in clinical circumstances for gastric-acid suppressing agents are PPI (Lundberg et al., 2008). PPI inhibit the irreversible proton pump function by targeting H^+/K^+ ATPase (Richardson et al., 1998).

H^+/K^+ ATPase is the last pathway for the acid secretion in the stomach (Richardson et al., 1998; Sachs, Shin, & Howden, 2006). The enzymatic pump secreting HCL and H^+ is inhibited by a potassium ion (K^+) – a competitive acid pump antagonist (APA) via binding to H^+/K^+ ATPase (Richardson et al., 1998; Sachs et al., 2006). PPI are considered to be weak bases that are able to cross cell membranes (Sachs et al., 2006). Activated by the acid environment through a second protonation at the parietal cells on the luminal side, the concentration increases to 1000-fold (Richardson et al., 1998; Sachs et al., 2006; Shin et al.; 1997).

H^+/K^+ ATPase is activated by a pH of $\leq 2.0 - 2.5$ while PPI has a pKa (acid dissociation constant/ value of acidity) of about 4 (Bhatt et al., 2010; Richardson et al., 1998). All PPI are activated by the second protonation from a H^+/K^+ ATPase proton and a present $pK_a \leq 1$ (Sachs et al., 2006). Reactions occur rapidly after the second protonation and are highly dependent on the value of acidity (Sachs et al., 2006).

The active form of PPI connects to cysteine residues to inhibit the acid-secreting pump (Sachs et al.; 1995). Besancon et al. (1997) tested three residues which were accessible for the different drugs. These results may give an idea to their effects (Richardson et al., 1998). Half-life for esomeprazole is around one hour but the suppression of gastric acid secretion can last up to 3 days (Bhatt et al., 2010; Sachs et al., 2006). Sachs et al. (2006) show that esomeprazole reaches a high concentration (steady state) fast, while in comparison to 20mg tenatoprazole, it does not have the same length of effect time. PPI have the ability to bind and dissociate from H^+/K^+ ATPase which is part of a long-lasting effect (Sachs et al., 2006).

In addition due to overutilization in PPI, the effect of antiplatelet drugs can be weakened (Montenegro et al., 2017). Antiplatelet drugs require an acidic environment for the gastric nitrosation (Bundhoo et al., 2011). A more alkaline environment can lead to less aggregation that can develop complications during surgery or an accident which makes it more difficult to take care of myocarditis or strokes (Montenegro et al., 2017). A reduced bioavailability of NO leads to less thrombocyte activation and supports these suggestions (Ghebremariam et al., 2014; Velmurugan et al., 2013; Webb et al., 2008).

To understand the bio activation of nitrate, which is one of the most researched molecules over the last years, a closer look to the existing pathways is needed (Bailey et al., 2012). The pathways to build the bio active form nitric oxide (NO) out of nitrate (NO_3^-) and nitrite (NO_2^-) are influenced among other things by the gastric pH. The effect of gastric pH on the bioactivation of nitrate will be tested with using PPI to decrease the gastric acid production and with that increase the gastric pH.

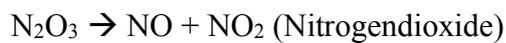
1.3. Pathways

One is the L-arginine–NO synthase (NOS) dependent enzymatic pathway. Ingested NO_3^- is reduced by bacteria secreted in the mouth by the salivary glands and their cells (Larsen et al., 2007). Formed nitrite (NO_2^-) is swallowed and NOS catalyzes NO_2^- to the bioactive NO under usage of O_2 by a 5-electron oxidation of L-arginine (Bailey et al., 2012; Bondonno, Croft, & Hodgson, 2016). Biological and mechanical stimuli activate NOS by a release of Ca^{2+} which forms a Ca^{2+} -calmodulin complex (Jin & Loscalzo, 2010).

This oxidation is necessary to accomplish normal physiological tasks and to ensure exercise tolerance. An impaired catalyzation can lead to a poor exercise tolerance and accompanying physiological problems (Lauer et al.; 2008).

The other pathway is NOS independent and the one researched in this study. Lundberg, Weitzberg & Gladwin (2008) investigated that the nitrate-nitrite-nitric oxide pathway gradually starts to work more while the oxygen tension falls (Bondonno et al., 2016). The study run under submaximal conditions and assuming a normal reaction of the body during exercise. The oxygen tension falls due to more O_2 consumption and more CO_2 production which leads to a hypoxic status (Lundberg et al., 2008).

Initially, the reaction starts the same way as the NOS-dependent pathway. The reduction by NO_3^- to NO_2^- is mainly initialized by the bacteria in the mouth secreted by salivary glands and predominately found on the dorsal surface of the tongue after the absorption of nitrate in the intestine (Bondonno et al., 2016; Larsen et al., 2007). After swallowing, NO_2^- enters the gastrointestinal tract and enters the circulation (Lundberg et al., 2004). Following the most important acidic reductions to form NO (Lundberg et al., 2008):



Not all of the swallowed nitrite is converted to NO. Around 75% of nitrate is transported via the kidneys and is excreted in the urine (Bondonno et al., 2016). Swallowed nitrite again enters the systemic circulation and enterosalivary circulation starts, which leads to a continued reaction of nitrite to NO to compensate high excretion by the kidneys.

Nitrite is reduced to NO in the acidic environment of the stomach (pKa 3.2, a value of acidity) (Lundberg et al., 2004). Transported through the circulation, NO is now used in the blood and tissues (Larsen et al., 2010).

Influence made by inorganic nitrate is essential to many different processes of the body and has a lot of different effects on the physiological system. Testing the influence of a PPI with esomeprazole as an active ingredient on the bioactivation of dietary nitrate while influencing the gastric pH seems to be appropriate as a test.

1.4. Research question and purpose

Since we cannot easily measure gastric pH and NO formation directly we use known indirect measures of NO formation.

- (1) Does the intake of PPI affect the oxygen consumption during submaximal exercise?
- (2) Do PPI influence the BP after taking a NaNO_3^- solution?

Lundberg et al. (1994) investigated that PPI almost abolished the NO in the expelled air and found evidence of the inhibition of gastric acid secretion. The pH- dependence and the influence was questioned (Lundberg et al., 1994; Lundberg et al., 2008). After being converted to NO, nitrate has several potential effects which are determined by the different biologically active substances surrounded (Bondonno et al., 2016).

The purpose of the study is to check if a higher gastric pH influences those effects. No current studies (as the author know by writing this thesis) deal with the influences that PPI have on NO activation during submaximal exercise

NO is an autocrine and paracrine inorganic anion. The half-life of NO is milliseconds while in nitrite it is 5-6 hours and is limited by scavenging reactions with hemoglobin (Hb), myoglobin or other radicals like for instance oxygen radicals (O_2^-) (Lundberg et al., 2008). This pathway is not oxygen dependent. A lot of other things can influence the secretion of NO and NO itself can influence reactions that are part of the pathway. This pluripotent characteristic is greatly enhanced by hypoxia or ischemic stress like during this study (Lundberg et al., 2008). Under basal conditions the metabolites and endogenous NO peaks after 60 minutes in the blood plasma (Jon O. Lundberg et al., 2004). Nitrate is now protonated under acidic circumstances in the stomach. Proton donator is the gastric acid HCL, that support NO to react spontaneously to nitrous acid (HNO_2). The presence of ascorbic acid (vitamin C) enhances the whole process with less secondary side products (Carlsson et al.; 2001). It is suggested that the combination of nitrate and vitamin C, in most vegetable nitrate-sources, enhances the whole conversion process. This suggestion makes the dietary intake even more important and interesting. Other nitrogen oxides are generated with nitrosating (N_2O_3) and nitrositing (NO_2) characteristics (Lundberg et al., 2008). N_2O_3 acts as a NO^+ donator to form a big variety of organic side groups and can form potentially N-nitrosamines (Lundberg et al., 2004).

N- nitrosamines are carcinogenic molecules (Tricker & Preussmann, 1991). As a result, exposure to carcinogenic substances from the diet or tobacco products need to be avoided. There are different ways to format endogenous N-nitroso compounds. One is through the acidic pH in the stomach (pKa 3.2) by using NO_2^- to form nitrous acids. As described before the NO^+ donation and as well the spontaneous formation of the nitrosating agents N_2O_3 and N_2O_4 take place in the stomach at the same time (Lundberg et al., 2004). This plays an important role when the gastric acid is reduced, for instance by PPI. Bacteria can colonize and mutagenize the gastric and cause diseases (Lundberg et al., 2004). There is still not enough evidence to say that there is a correlation between high nitrate intake and gastric cancer but it can be related to vitamin C and other antioxidants intake through nitrate-rich vegetables (van Loon et al., 1998). Furthermore, an enhancing effect of vitamin C and other phenols to the reduction of NO_2^- to NO is investigated.

2. Methods:

2.1. Subjects

The randomized, double-blinded, placebo-controlled, crossover study ran with 6 healthy, active and non-medicaments taking adult subjects (mean \pm SD, age 29 ± 5 years, height 170 ± 5 centimeters, weight 70 ± 5 Kg, BMI $24,36 \pm 1,75$ Kg/m², blood pressure $119/ 77 \pm 6$ mmHg, 3 male and 3 female). All the participants were informed about the procedures during the study, with none of the participants showcasing a high blood pressure (WHO, 2018-08-03).

The procedures were approved on October 25th, 2016 by the regional ethics committee in Stockholm. The subjects gave their written informed consent during the first meeting in the laboratory where everything was explained, and open questions were answered.

Furthermore, information and risks were provided, and the benefits of the study were also explained. A list of nitrate rich foods was handed over and the time, data and number of pills were written down and a reminder was sent via Facebook messenger, 24 hours before the next occasion to take the pills.

Subjects had to rest at least one week before the next meeting, then a further 3 pills were distributed after the second meeting. Whether me as investigator nor the subject knew which kind of treatment (placebo or esomeprazole) was taken during which time.

2.2. Procedures

Subjects were selected through “Studentkaninen”, Facebook, the student presentation at the university and word-of-mouth communication. Information was spread via an information sheet which was sent per e-mail or Facebook-messenger when someone was interested.

Before testing, all technical parts were calibrated, and all used surfaces were cleaned with 70% ethanol. With regards to the test, used materials were organized and then collected.

During the first meeting, the subject was provided with all the necessary information and was able to ask questions as well as being given the possibility to decline without giving reasons at any time during the study. After signing the written consent, the participant was measured, weighed and had their blood pressure taken. Additionally, the manually braked pendulous ergometer cycle (828E, Monark Exercise AB, Valsbro, Sweden) was adjusted, fitting to the subject’s height. As criterium, the subject had to sit upright with their torso and knee slightly

flexed in the lowest position. It was important to have the same saddle height during each test as well as making sure that the handlebar was suitable for the subject's needs.

To make this experiment valid and reliable everything was recorded. The size of the oxygen mask (7450 series V₂Mask™, Hans Rudolph, Inc., Kansas City, USA) was recorded and written down. After warming up on the bike, the subjects were able to test the feeling of the mask and the different stages for the test. A VO_{2max} was provided to gather more participants and acted as a reward for their invested time. None of the values of this test are mentioned or even analyzed in this thesis.

Followed the timeline of all the testing procedures:

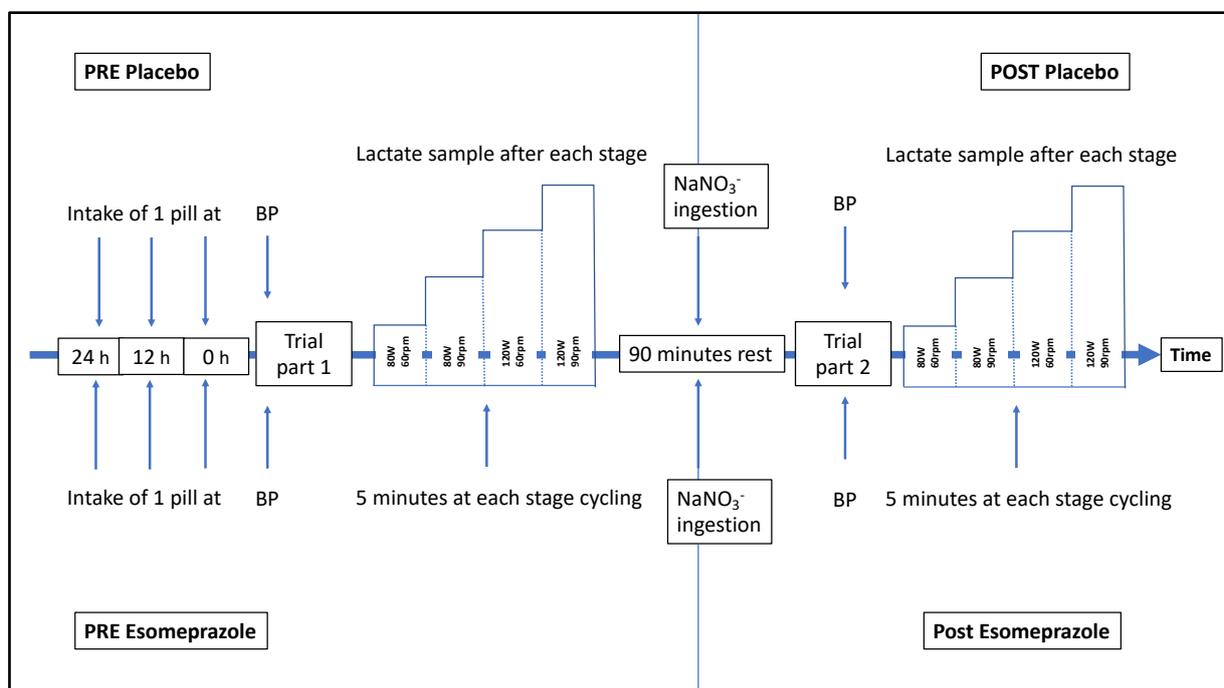


Figure 1 Timeline for testing trial 1 and 2

Monark Ergomedic 828 E uses a break belt system to control the power output. To change the workload, the pedal rate was changed, or the workload knob adjusted. The Kilopond (KP, metric unit of force) was measured by a flywheel.

GIH created a table with Monark to convert KP into Watt and make it reproducible for other used metrics of power. 32 Watt with a 60RPM are equal to 0,5 KP and 64 Watt with 60RPM was similar to 1,0KP (GIH, Conversion of Kilopond to watts).

The cadences of the tests were chosen to simulate a lower and a higher span of what can be considered a normal cadence for recreational cyclists and submaximal workload (Abiss et al., 2009).

The different values of KP to reach different outcomes was used to determine and reach a steady state of the ventilatory components measured, which on one hand were high enough to raise the ventilatory values and on the other hand not too high to overcome a steady state and induce fatigue.

The scale on the bike had just half-kilopond steps. Therefore, the scale was completed with 0,1 steps by a water-resistant marker to make it more accurate and repeatable. To find the correct value of KP during the four stages, testing with different numbers to get a relatively equal value of Watt for each stage and rpm was conducted and based on the formula:

Power (Watt)= force (kilopond) x frequency of rotation around a fixed axis (RPM), the KP was estimated.

The subject was provided with the first of two randomly assigned pills after the first information meeting. Following this, a date for the next occasion was made and instructions that included the exact time, date for taking the pills and when to start avoiding nitrate-rich food (an information sheet was provided). The pill contained either 10mg of the active agent esomeprazole or 10mg of sodium chloride (placebo).

Subjects were informed of the purpose of the study due to the ethical aspects and risks of esomeprazole by raising the gastric pH (more basic) and a possible influence on the body. To make sure the test was valid and reliable, the steps were exact the same for the first and second test trial that followed. The ear was prepared with Finalgon® to encourage the blood flow for the later used capillary blood selection.

It was important for the subjects to feel comfortable and less stressed to produce a valid value for the blood pressure. Subjects had to rest at least three minutes before their BP was taken. The BP was taken with a digital automatic blood pressure monitor (OMRON Medizintechnik GmbH, MIT Elite, Mannheim, Deutschland) on their left arm, three times. Between every measurement, the blood pressure cuff was loosened for 60seconds to make sure that the blood flow went back to normal. Vinyoles et al. described in 2014 that waiting or not is interchangeable and does not play a clinical role.

After taking the venous blood samples (40ml with an eclipse blood collection needle by BD Vacutainer®, Pittsburgh, USA), subjects were either allowed to rest (if they were more

sensitive to blood giving) or able to start warming up on the ergometer. During this time the blood sample was centrifugated with an Eppendorf Centrifuge 5810R (Leipzig, Germany). After taking the blood samples, the first part of the trial was initialized. The oxygen mask and all technical devices were checked, and the first stage started with 60RPM (Revolutions per minute) with 1,3KP which is equal to 80Watt. The subject's heart rate (HR) was measured throughout the process with a Polar FX1 and the H1 sensor (Polar Electro Oy, Kempele, Finland). During the test phase, the HR and the shown pulmonary gas exchange rates were observed to make sure the patient was healthy and feeling well, and at the same time if everything was working according to the protocol. Each stage took five minutes to reach a steady state and a capillary blood selection was taken before a new stage was entered (Whipp & Wasserman, 1972).

Capillary blood samples were taken with a Finalgon[®] prepared earlobe. Finalgon[®] remains were removed to avoid contaminating the sample from the earlobe. After removing the area around the earlobe, it was disinfected with swabs for skin cleansing (B Braun, Melsungen, Germany). During the whole procedure, the investigator (me) wore milky sensitive nitrite gloves (Papyrus, Stockholm, Sweden).

The earlobe was punctuated using safety lancets (Sarstedt extra 18G, Nümbrecht, Germany) for capillary blood sampling with a penetration depth of 1,8mm. The first blood drop was brushed away to avoid tampering with the sample. 20 μ L end to end plastic capillaries (Sodium [Na⁺] heparinized) were filled with blood and put into a glucose/lactate hemolyzing solution (1 ml, EKF- diagnostic GmbH, Barleben, Germany). They were analyzed immediately after the first test. After the last stage of the first part, the subject got a solution of water and 10mg sodium nitrate (NaNO₃⁻) per kg bodyweight. This dose was established from previous studies to show an effective reduction of blood pressure and VO₂ at submaximal working rates (Clements et al.; 2014). The used NaNO₃⁻ was commercially distributed Salpeter (Santa Maria, Göteborg, Sweden) and dissolved in tap water. After the solution had been consumed the resting time of 90 minutes began. Sinead et al. 2018 wrote that it takes approx. two hours for nitrate to reach a peak in the blood plasma depending on the circumstances and the individual. While waiting for the second bound the subjects were allowed to drink water but not to eat or doing any exhausting physical activities.

During the 90 minutes I began pipetting the centrifugated blood sample. The blood serum was transferred via a one-time use pipette to a small test-tube. Close attention was paid to transferring the plasma without any tracks of the serum. The test-tube was labelled and frozen

at -26°C (Siemens, München). Furthermore, the lactate was also analyzed with Biosen C-line Clinic (EKF Diagnostics, Cardiff, UK). The Biosen glucose and lactate analyzer uses a special chip sensor technology which provides the glucose and lactate values with less than 2% CV (capacitance voltage profiling).

Measuring with a range starting at 0.5 mmol/L some results were not available due to too small concentrations. The biochemical process uses the reaction of β -D Glucose and L Lactate with the immobilized enzymes glucose oxidase/lactate oxidase. Resulting reaction products (pyruvate and hydrogen peroxide) detecting to an electrode (just hydrogen peroxide). The resulting current flow is proportional to the glucose- and lactate concentration (EKF Diagnostics, Manual Biosen C-Line, 07/2013).

Any questions the subject had were discussed during the break. BP was measured once and noted after 90 minutes of rest. A second blood sample was taken by authorized staff and the exact same procedure as in part one was done again.

While the subject was resting the oxygen mask was disinfected, the technical material was calibrated, and everything was arranged like it was before the first bound. After taking the blood sample to centrifugate, the subject started with the same protocol and all the different parameters were recorded, as well as the taking of lactate samples.

After the last stage, the subjects were provided with the second part of the pills. Neither me as investigator nor the subject knew which part of the pills was provided. The pills were always labelled with A and B and the number of the subject to know when the keycode of the pills was provided, when which treatment was given.

The next date for the final meeting was set and a schedule for taking the pills was arranged. In addition, subjects were reminded to do the same thing before the next occasion to achieve the best results.

Finally, the last step was analyzing the lactate and cleaning and disinfecting everything. All results were noted in a laboratory journal.

2.3. Measurements

All measurements for the ventilatory values were conducted with the Oxygen Pro[®] (Erich Jaeger GmbH, Hoechberg, Germany). Before each test session ambient conditions were measured with a portable humidity and temperature instrument named the HygroPalm 0 (Rotronic, Crawley, West Sussex, UK).

The calibration procedures included the control of low and high flows with a built-in automatic calibration system. Gas-analyzation was accomplished with a high precision gas mixture (15% O₂ and 6% CO₂) provided by Air Liquide AB (Kungsängen, Sweden) and the ambient air in the laboratory. The breath-by-breath program delivered a continuous measuring of the ventilation and the concentration of the expiration gas by the subject just outside the mouth (Rietjens et al., 2000) The pulmonary gas exchange and ventilation were saved every 15 seconds during the test.

Lactate and glucose samples were taken 20 seconds after a new stage was entered with a capillary tube. The heart rate was displayed with a short-rated radio telemetry (Polar F1, Polar Electro Oy, Kempele, Finland). The principle is based on an ECG (electro-cardiogram) which is found in the medicine. Later, the detection of electrical signals of the heartbeat and transmitting the data to the training computer was conducted. The training computer calculate the number of beats per minute (bpm) by reading the transmissions of the heart rate sensor. Before and after each meeting, all used materials and surfaces were cleaned with 70% ethanol to keep the risk of contaminated samples and test outcomes as low as possible.

2.4. Data analyzing procedures

The test of normality (t-test) ran for BP, VO₂, and VCO₂ with SPSS[®] (SPSS Inc., Chicago, Illinois, USA, Version 24.0.0.0) with a 95% confidence interval. Kolmogorov-Smirnov and Shapiro Wilk tests were used.

Breath by breath data during each meeting and each test was analyzed by building the mean over every minute of each stage. Minutes 4 & 5, 9 & 10, 14 & 15 and 19 & 20 were taken to get the mean. Whipp & Wasserman (1972) investigated the reach of a steady state for the ventilatory values VO₂ and VCO₂ after 3 minutes. The protocol used four stages to simulate the same procedure while having a constant work rate at each point. Former study outcomes

could be used as a basic consideration for choosing the right protocol (Whipp et al.; 1982; Whipp & Wasserman, 1972).

Every 15 seconds the Oxygen Pro[®] captured the data. The conversion of the used minutes in seconds enabled a much more manageable continued use.

Table 1 Conversion minutes in seconds of the last two minutes at each stage

Minutes	Seconds
4/5	195-300
9/10	495-600
14/15	795-900
19/20	1095-1200

The mean for each stage and the analyzed data was simplified and gathered together in one table. The BP systolic/diastolic in mmHg, VO₂ in ml/min, RER, VE in ml/min, the VCO₂ in ml/min, the Lactate in mmol/l, the Glucose in mmol/l and Heart Rate (HR) in bpm were divided by pre and post NaNO₃⁻ intake and the four different stages. The constructed table showed the best review of the collected data, including the standard deviation (SD).

In addition, the VO₂, RER, VE, VCO₂, Lactate, and Glucose were also divided by the four different work-power stages, 60rpm/ 1,3KP and 80 Watt; 90rpm/ 0,9KP and 80 Watt; 60rpm/ 2,0KP and 120 Watt and 90rpm/ 1,3KP and 120 Watt. Independent samples t-tests (SPSS) were also used for comparing means on the given confidence interval.

3. Results

This thesis is part of a bigger project of GIH and KI in Stockholm and can be considered as a pilot study. All measurements made by the author can be considered as to be continued.

Besides knowing the effects of nitrate on VO_2 and BP throughout former studies (Larsen et al., 2010; Montenegro et al., 2017), measuring VCO_2 , VE, RER, Lactate, Glucose and HR was to see if any effects of PPI ingestion are shown on those values as well.

Depending on if the sample size (number of subjects) and the effect size (e.g. the difference/expected difference in the outcome of the two groups) increase, the power would increase as well.

The current study is under powered due to a lack of participants. A priori calculated with G*Power (Version 3.1.9.3), 10 to 16 subjects would have been necessary to get an effect of 2-3 %. A lack of time (each trial took approx. 3 hours) or the circumstances that there was not reward given in form of financial support could be used as an explanation. The Participants who took part in the study were aged 29 ± 5 years, 170 ± 5 centimeters tall, weighed 70 ± 5 Kg, had a blood pressure of $119/77 \pm 6$ mmHg and were divided by 3 female and 3 males (mean \pm SD).

Differences in the treatment with esomeprazole and placebo are shown in the BP, VO_2 and VCO_2 without statistical significance. Significant differences were reported in the post nitrate ingestion between female and male in general. As well as differences in the placebo treatment before the nitrate ingestions between female and male.

A closer look at the outcome of the study was taken at the differences in the treatment with esomeprazole and placebo after ingesting the nitrate solution (10 mg NaNO_3^- per Kg bodyweight with water). This helped to answer the research question, if there is an effect on the measured values in post nitrate intake with esomeprazole that would be, based on former study's, because of the change of the gastric pH.

Post values were analyzed with SPSS® and an independent sample t-test calculated the differences between pre and post nitrate solution ingestion for the different treatments and the differences in post nitrate ingestion in esomeprazole and placebo treatment. The research question; if PPI (esomeprazole) influence nitrate bioactivation while increasing the gastric pH, can be explained by using the parameters blood pressure and oxygen uptake which were

detected to be influenced by NO in several studies (Larsen et al., 2007; Lundberg et al., 2008).

All the values were rounded to two decimal figures in the text. During the analyzing process all given decimal places for t-values were used.

For the statistical analysis $\alpha = 0.05$ with statistical significance proven by $t \leq \alpha$, was assumed.

Blood values could not be used for the results because they were not analyzed in time.

3.1. VO_2

The numbers 1, 2, 3 and 4 describe the four different stages during the test (minutes 1-5, 6-10, 11-15 and 16-20).

Mean values for post VO_2 were 1765,36 ml/min (SD \pm 271,22; Esomeprazole) and 1708,95 ml/min (SD \pm 291,71; placebo). Significance was denied with $t = .491$.

A coherence between taking esomeprazole or the placebo and a higher consumed amount of oxygen during the four stages was shown.

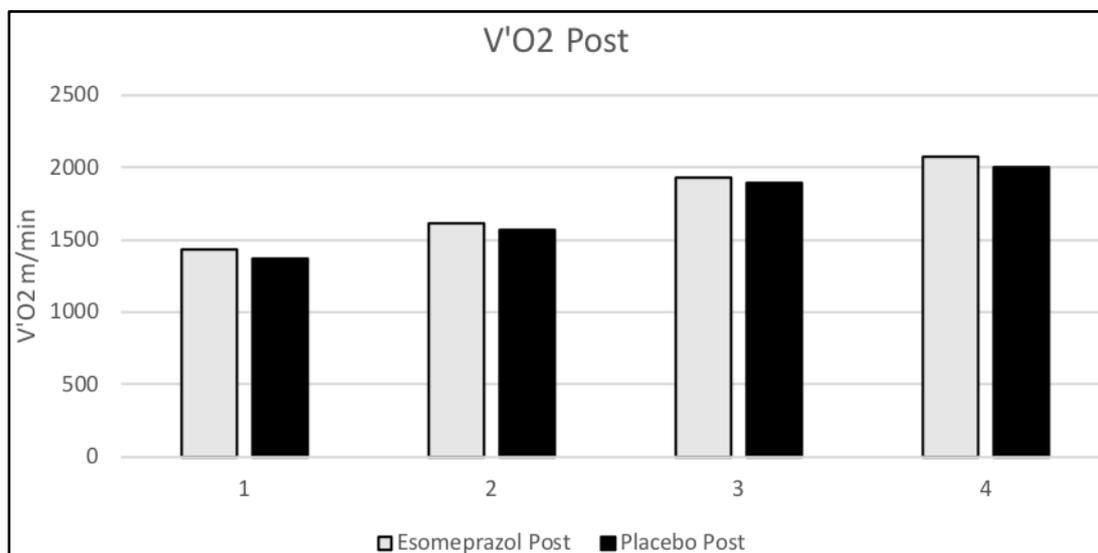


Figure 2 VO_2 post $NaNO_3^-$ ingestion

Differences were showed without significance in post nitrate ingestion VO_2 esomeprazole female ($1755,85 \pm 279,43$ ml/min) and male ($1774,86 \pm 274,82$ ml/min) with $t=.868$, post nitrate ingestion placebo female ($1674,53 \pm 260,60$ ml/min) and male ($1743,37 \pm 327,75$ ml/min) with $t=.762$.

The oxygen uptake in female increased by 4,63% when taking esomeprazole while male was 1,77% lower when taking the placebo.

3.2. Blood pressure (BP)

No significant differences between systolic BP esomeprazole (120,17 mmHg; SD \pm 5,88) and systolic BP placebo (114,67 mmHg; SD \pm 3,88) with $t= .085$. Diastolic BP esomeprazole (75,17 mmHg; SD \pm 7,55) and placebo (73,58 mmHg; SD \pm 2,91) showed no significant varieties ($t= .642$). Groups divided by gender had no significant difference in values post nitrate ingestion, whether systolic BP ($t= 0.85$) nor diastolic BP ($t= .607$).

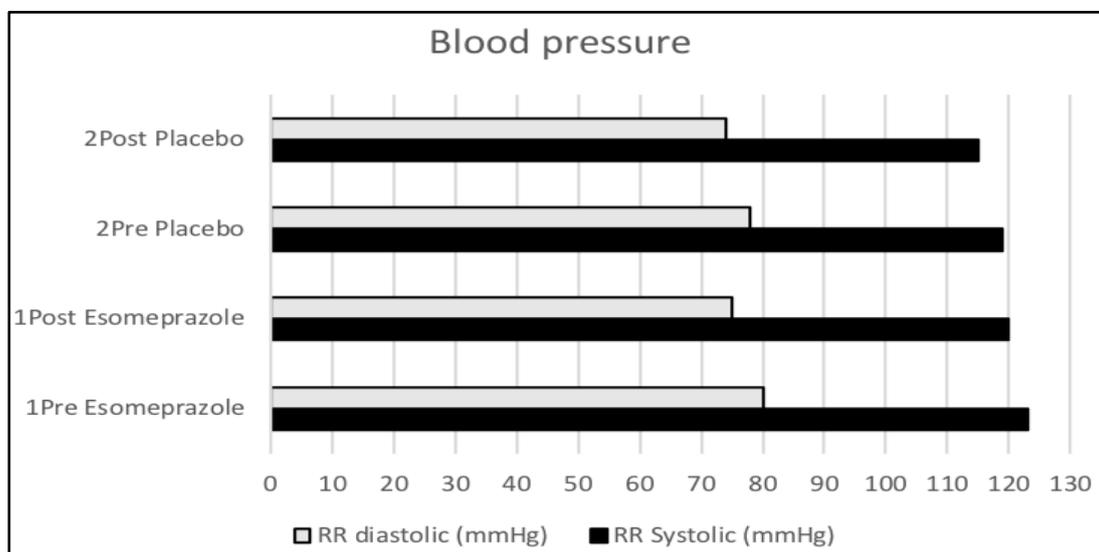


Figure 3 BP post NaNO_3^- ingestion

Differences were visible between pre-esomeprazole/ pre-placebo and between post-esomeprazole/ post-placebo but not significant.

Due to the earlier described underpowered study, there were no significant results except for the RER, VE and HR. Those significant values were gender specific.

3.3. RER

The respiratory exchange ratio (RER) value with esomeprazole was 0,89 (SD \pm 0,04) and the value with the placebo was 0,91 (SD \pm 0,05). No significance with $t= .115$.

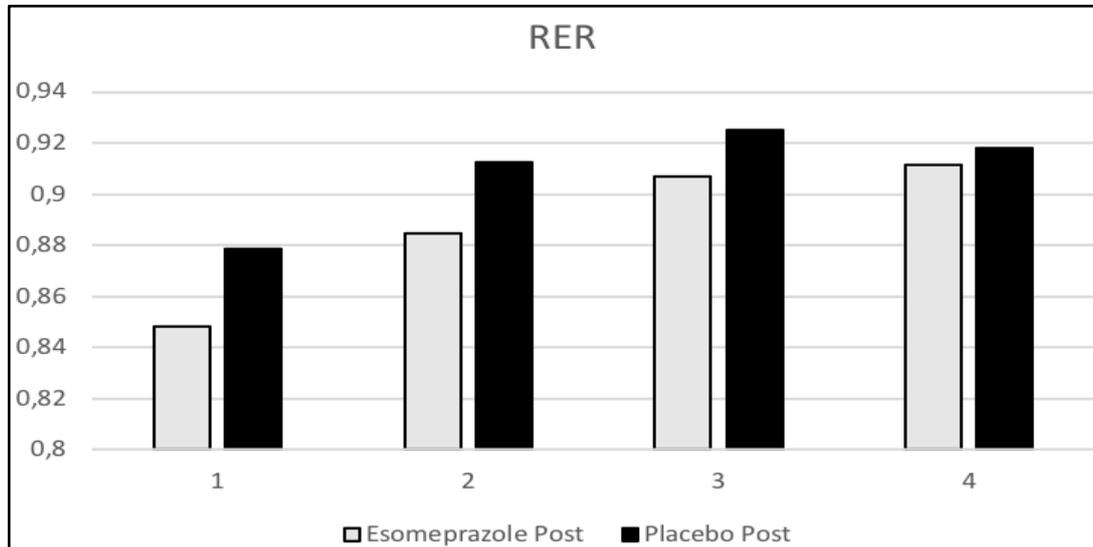


Figure 4 RER post NaNO_3^- ingestion

There was a difference between the two treatments shown. Significant differences in post nitrate ingestion values investigated by the group variable gender. Female 0,88 (SD \pm 0,05) and male 0,92 (SD \pm 0,38) with $t= .006$. Pre-values (before nitrate solution ingestion) divided by the group variable gender female 0,93 (SD \pm 0,05) and male 0,93 (SD \pm 0,05) with $t= .744$. No statistically significant difference between female (0,87; SD \pm 0,4) /male (0,09; SD \pm 0,04) esomeprazole Pre-nitrate ingestion ($t= .138$), but a significant detail between pre-female (0,89; SD \pm 0,05) and pre-male (0,93; SD \pm 0,30) placebo treatment ($t= .015$).

3.4. VE

Values for VE were determined with esomeprazole by 45,41 L/min (SD \pm 9,78) and placebo treatment with 43,95 L/min (SD \pm 7,65). Significance was not given with $t= .568$.

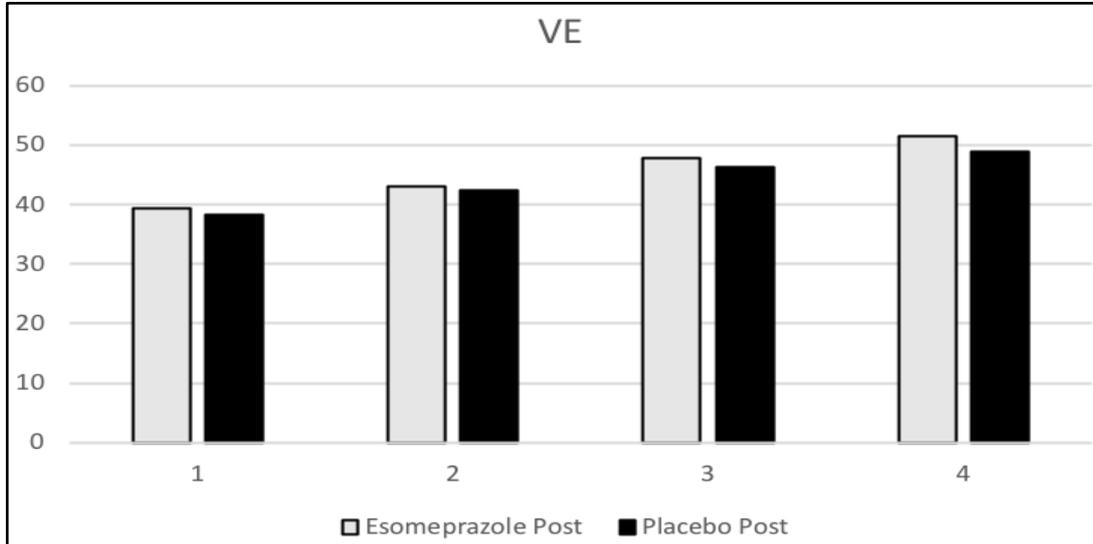


Figure 5 VE post NaNO_3^- ingestion

A slightly decreased VE after ingesting the nitrate solution with the treatment placebo was observed. A significant difference between female ($40,79 \pm 7,20$ L/min) and male ($50,03 \pm 10,09$ L/min) pre-nitrate ingestion with esomeprazole ($t= .017$, 22,65% difference) while there was no significance ($t= .946$) between female ($44,06 \pm 7,77$ L/min) and male ($43,84 \pm 7,88$ L/min) pre-nitrate ingestion with the placebo treatment (0,5% difference).

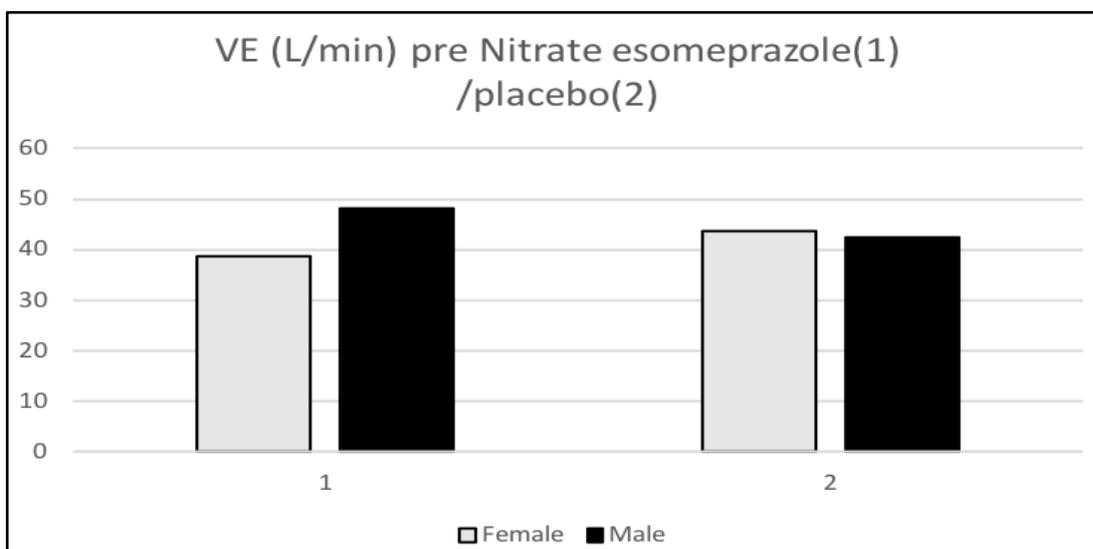


Figure 6 Sex-difference pre- NaNO_3^- ingestion in VE divided by treatment

3.5. VCO_2

No significance shown for post VCO_2 -values. Esomeprazole ($1641,67 \pm 320,34$ ml/min) and placebo ($1534,12 \pm 264,92$ ml/min) with $t= .211$.

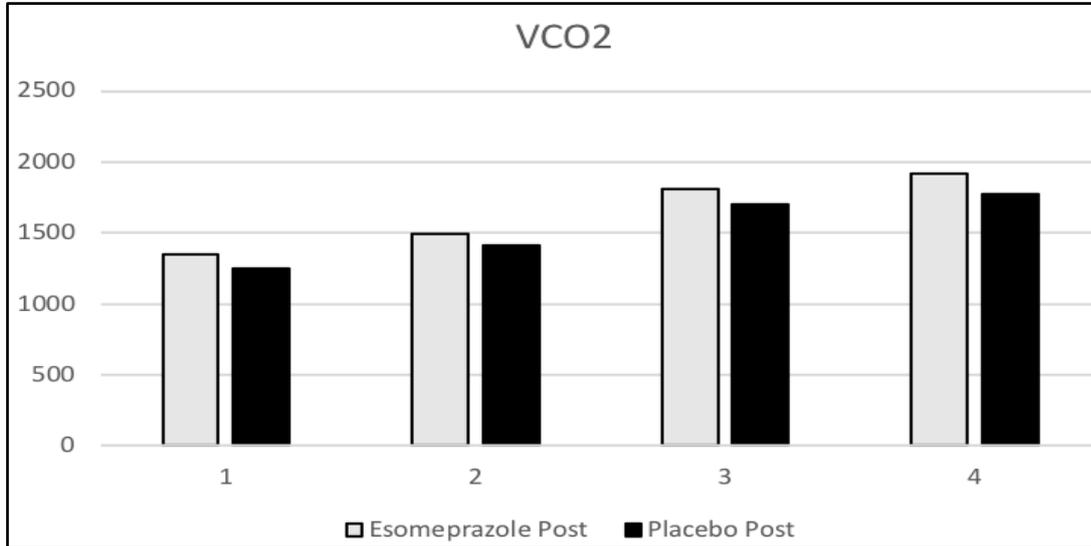


Figure 7 VCO_2 post $NaNO_3^-$ ingestion

More VCO_2 exhaustion is recognized when taking esomeprazole in comparison to the placebo.

Gender differences without significance in post nitrate ingestion esomeprazole female ($1662,13 \pm 345,15$ ml/min) and male ($1621,22 \pm 307,44$ ml/min) with $t=.575$, post nitrate ingestion placebo female ($1466,73 \pm 172,32$ ml/min) and male ($1601,52 \pm 327,33$ ml/min) with $t=.220$. Female VCO_2 increased by 11,76% when treated with esomeprazole while male increased by 1,22%.

3.6. Glucose

Slight differences in glucose values (esomeprazole $3,23 \pm 0,44$ mmol/L and placebo $3,13 \pm 0,46$ mmol/L) but no significance ($t= .420$).

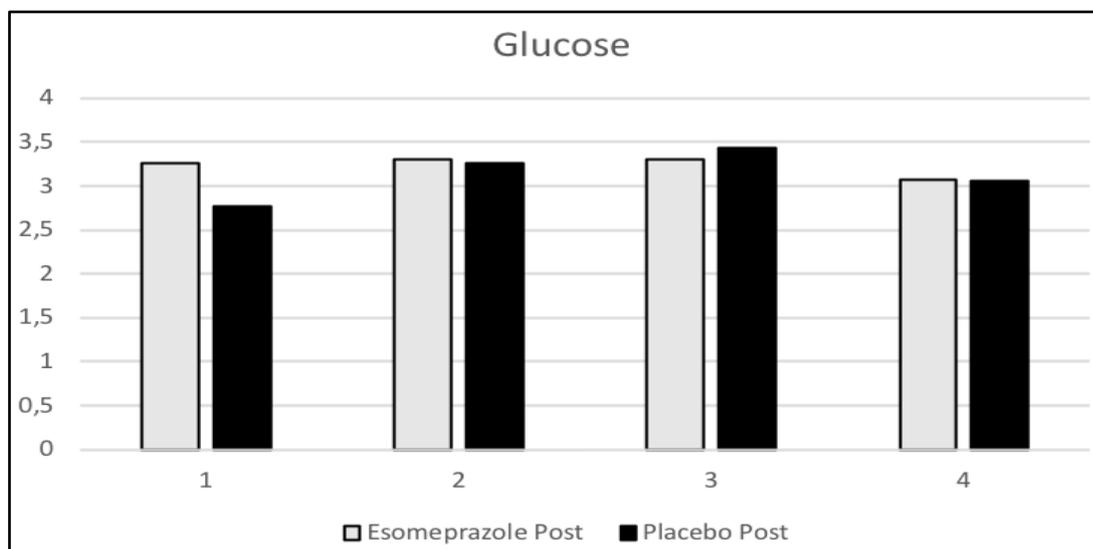


Figure 8 Glucose post NaNO_3^- ingestion

3.7. HR

No differences in the heartrate (HR) post nitrate solution intake. 122,70bpm (SD $\pm 20,01$) with esomeprazole treatment and 117,85bpm (SD $\pm 17,78$) with placebo, $t= .380$.

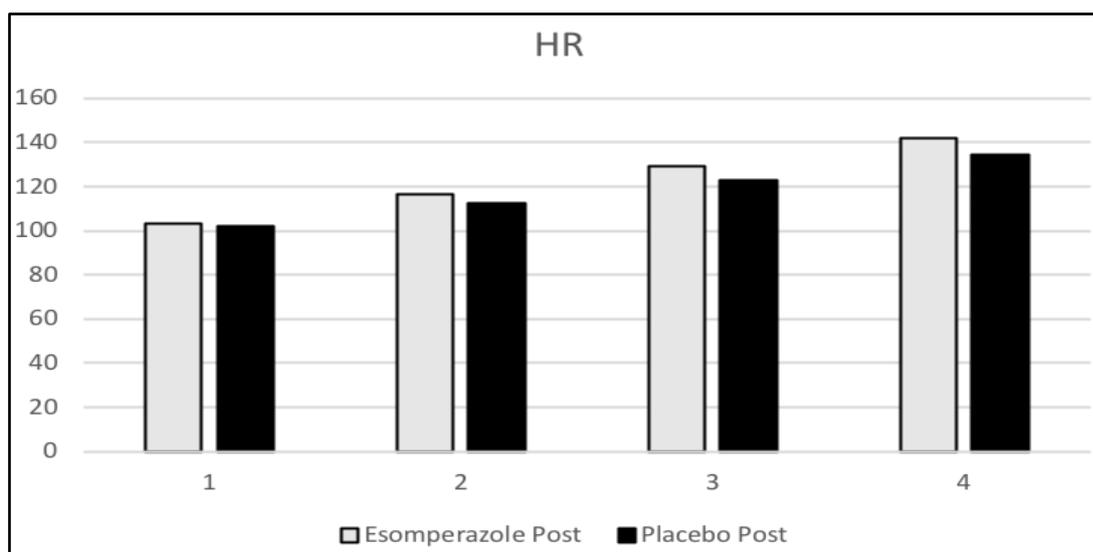


Figure 9 HR post NaNO_3^- ingestion

Significant differences in heartrate with $t= .000$ while woman 130,12bpm (SD \pm 18,60) and men with 110,43bpm (SD \pm 13,44) post nitrate ingestion. Pre-values female 128,73bpm (SD \pm 18,88) and male 110,52bpm (SD \pm 12,24). Significance proved by $t= 0.000$.

All occasions with pre/esomeprazole (female: 133,27bpm/SD \pm 20,21; male: 112,13bpm/SD \pm 13,60; $t= .007$), pre/placebo (female: 126,97bpm, SD \pm 17,12; male: 108,74bpm, SD \pm 13,66; $t= .009$) were significant.

3.8. Lactate

Lactate- values with esomeprazole reached 0,71 mmol/L (SD \pm 0,54) while the value with the placebo was 0,61 mmol/L (SD \pm 0,56).

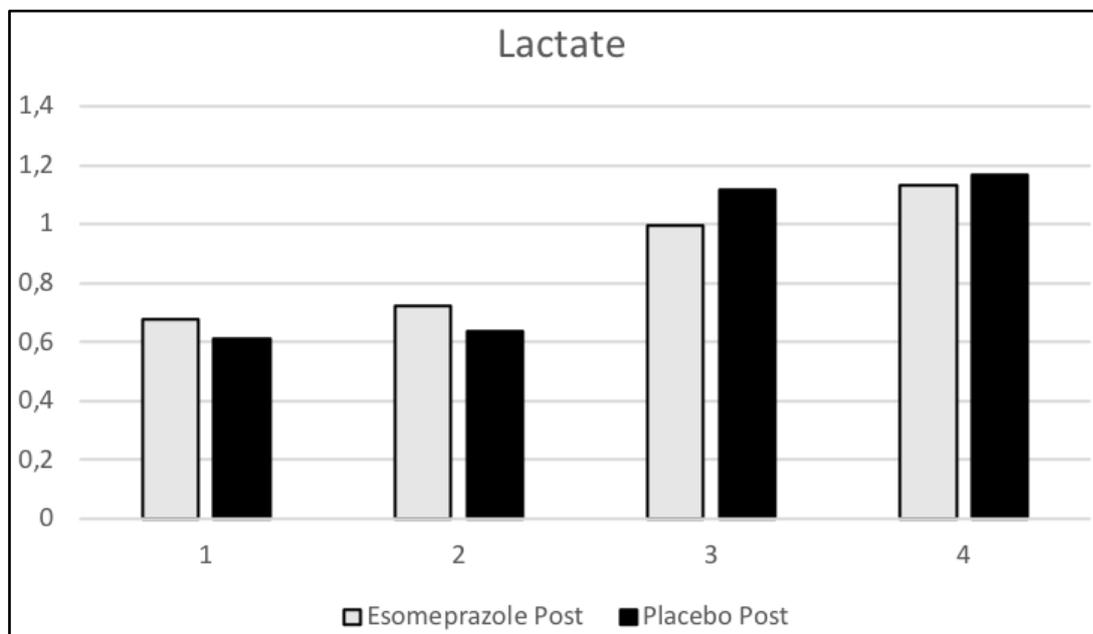


Figure 10 Lactate post NaNO_3^- ingestion

A continuous increase of lactate values can be displayed. No significant difference with $t= .534$.

4. Discussion

The research questions if the intake of PPI affects the oxygen consumption during submaximal exercise and do have PPI influence on the BP after taking a NaNO_3^- solution can just be answered with tendencies of an effect or influence. Statistical significance is not given.

Purpose of the study was to show that if the gastric pH is influenced by taking a PPI, the bioactivation of nitrate should be smaller and so the positive effects like lowering the oxygen costs and lowering the BP can't be answered for sure. More testing is needed with more subjects. Differences are shown in VO_2 and BP in post values between esomeprazole and placebo intake.

Following considerations of the results and deliberations what this pilot study may lead to.

VO_2 reflects the oxygen consumption during exercise (Basu et al., 2008; Grassi et al., 1996). The ingestion of NaNO_3^- improves the efficiency of the oxidative metabolism (Bailey et al., 2009). VO_2 increased by 2,62% from pre to post nitrate ingestion with esomeprazole intake and increased 0,11% with the placebo. The effect is measurable. Different explanations can be used. Firstly, the theory that NO_2 can replace oxygen (O_2) as an electron acceptor during the respiration (Basu et al., 2008). Reducing the O_2 cost with nitrate intake from 1765,36 ml/min (SD \pm 271,22; esomeprazole) to 1708,95 ml/min (SD \pm 291,71; placebo) represents a decrease of 2,52% during the submaximal effort (Bailey et al., 2009). Oxygen delivery and oxygen utilization is displayed with VO_2 (Itoh et al., 2013). Directly connected to VO_2 is VCO_2 . VCO_2 shows how much CO_2 is exhaled. The study shows no significant difference between VCO_2 esomeprazole ($1641,67 \pm 320,34$ ml/min) and placebo ($1534,12 \pm 264,92$ ml/min) intake after NaNO_3^- solution ingestion. Recognizing that during esomeprazole treatment, VCO_2 increased by 1,68% in comparison to that day baseline value while VCO_2 in placebo treatment decreased by 0,24% in comparison to the testing day baseline. It is interesting to see that there is an effect and a connection to the values of VCO_2 . Both esomeprazole treated values (pre/post nitrate ingestion) are higher than those treated with placebo. This can be caused by the effect of PPI or the general influence of respiration, circulation and metabolism during the test (Itoh et al., 2013).

Those values might be influenced by the functions of the mitochondria. Clerc et al.(2007) suggested that NO might reduce the slippage of mitochondrial proton pumps which would lead to an enhanced efficiency of the oxidative phosphorylation. This effect can be enhanced by a larger number of reduced slippages by more mitochondria which will be built through training and can enhance the effect of O₂ utilization (Jacobs & Lundby, 2012). Sheel et al. (2004) investigated that men tend to have lower ventilation (VE) during exercise and are the cause of existing sex-differences in the respiratory response. Outcomes of the study showed a 22,65% difference between female and male pre-nitrate intake with esomeprazole while there was just a 0,5% difference with the placebo which shows an influence of the PPI and as well a sex-difference.

Another point is the bioactivation of NO which includes the conversion from NO₃⁻ to NO₂⁻ to NO under specific physiological circumstances (Benjamin et al., 1994). Those can change by taking drugs (PPI). PPI are the worlds most prescribed drug. In the study used esomeprazole is the third most taken drug in the USA (Boparai, Rajagopalan, & Triadafilopoulos, 2008; Chowdhry et al., 2018). A chronic exposure to PPI might enhance the prevalence of major cardiovascular events by impairing the vascular homeostasis and reduce the benefit of oral nitrates (Ghebremariam et al., 2014).

Whereas ageing is considered to slow down the bioactivation of NO by endothelial dysfunction and reduce the red cell mass (Fisher & Hollenberg, 2006; Lauer et al., 2003; Loscalzo, 1995; Murphy, 2014). With knowing that, a prescription of PPI should be even more thought through.

More a gender specific difference between female and male is shown for the treatments. The red cell mass represents the ratio of hemoglobin which we know influences the reaction to NO out of NO₂⁻ (Prommer et al., 2008). Different levels of hemoglobin can also be an explanation for the significant difference in heart rate between the female and male individuals that participated. For instance, the female had 130,12bpm (SD ± 18,60) and male had 110,43bpm (SD ± 13,44) post nitrate ingestion. Pre-values female were 128,73bpm (SD ± 18,88) and male were 110,52bpm (SD ± 12,24). The difference is significant.

Recent studies questioning if those differences could be used to make new formulas for calculating the maximum heart rate to be more reliable and valuable throughout different tests when both sex are involved (Gulati et al., 2010; Sydó et al., 2014). This suggestion leads to the hypothesis, if there are differences in venous blood samples in hemoglobin between female and male, it might be possible that these values lead to different NO levels and provide a different

effect among female and male testing subjects (Ganji & Kafai, 2009; Garn, Smith & Clark, 1975; Vahlquist, 1950; Yip, Johnson & Dallman, 1984). The effect of nitrate ingestions and the influence of hemoglobin (Hb) to transfer the oxygen (O₂) to the tissues also depends on the iron (Fe) in the red blood cells (Lundberg et al., 2008). Studies showed that females have 12% less iron premenopausal than male subjects of the same age, while females have the same level pre- and postmenopausal (Ganji & Kafai, 2009; Garn et al., 1975; Rege, Brookins & Fisher, 1982; Tilling et al., 2012; Vahlquist, 1950; Yip et al., 1984). To support this suggestion VO₂ in female subjects raised by 4,63% when taking esomeprazole in comparison a decrease of 1,77% on the male side under the same conditions. In addition, the baseline in male was higher (1743,37 ± 327,75 ml/min) than in female (1674,53 ± 260,60 ml/min) when taking the placebo. Those gender-specific differences might be influenced by a higher androgen level in male and a higher estrogen level in female which causes an inhibitory effect in women on building red cell mass (Jelkmann, 2011; Shahani et al., 2009). These studies also show that the results may depend on where a woman is during her menstruation cycle or at least to ascertain their hormone levels via a blood analysis.

Another difference with regards to gender is shown in the metabolism of female and male subjects (Tarnopolsky, 2000). We assume a constant RER (respiratory exchange ratio as a definition for the metabolism of the different macronutrients) throughout the whole test (t= .115) (Bordoni & Capozzi, 2014). Between the pre-nitrate ingestion female and the pre-nitrate intake male subjects, with the placebo treatment, there is a significant difference with t= .015 shown. Esomeprazole also seems to have a different effect on the metabolism in female and male. The difference in post-nitrate ingestion between sex (t= .006) reinforces the variability in the metabolic response (Bordoni & Capozzi, 2014). A tailored intake of nitrate for each sex and life-situation can be discussed on the basis of those outcomes (Bordoni & Capozzi, 2014). Multiple variations of nutrigenetics may influence the well-being of each individual depending on their metabolic footprint (Bordoni & Capozzi, 2014). Up- and down regulations also explain a variety of vitamins and minerals and could be influenced by the digestibility and bioactivation of the ingested nutrient (Bordoni & Capozzi, 2014). NaNO₃⁻ (sodium nitrate) used in this study improves the efficacy of muscle oxidative metabolism differences in sex as we can see on the RER changes (Bailey et al., 2009). Different studies show different effects of different nitrate sources. Nitrate-rich vegetables as beetroot or spinach and their juices show a greater effect than capsulated NO₃⁻ salts (Ashworth et al., 2015; Jonvik et al., 2016; McDonagh et al., 2018; Wylie et al., 2018). More compounds in beetroot juice like

Vitamin C or polyphenols enhance the NO production and contribute to the pathway (Bailey et al., 2009; Flueck et al. 2015; Peri et al., 2005; Rocha et al. 2009 . Morgado et al. (2016) tested a beetroot gel to increase the nutritional strategy of bio accessibility of potassium and antioxidants. Clinical studies show that the best benefits for cardiovascular health are with 5,5 to 6,4 mmol/L potassium per 200g of a concentrated gel (Kapil et al., 2015; Kapil et al., 2010; Morgado et al., 2016). Another group of scientist tested a beetroot gel based on this data with $6,3 \pm 0,41$ mmol/100g with positive effects (da Silva et al., 2016).

Additionally, studies showed that concentrated beetroot juice (70-140ml) has the biggest effect among other substrates like sodium nitrate (McDonagh et al., 2018; Wylie et al., 2013). Some studies showed a significant effect using dietary nitrate solutions with potassium or sodium nitrate (Bailey et al., 2009). Others had no significant results.

Supplements with nitrate may have a possible decrease of 20% of the O₂ costs (Bailey et al., 2009). The O₂ consumption did neither with placebo nor esomeprazole decrease significant. It is shown that the placebo leads to a small increase (0,11%), while esomeprazole leads to 2,62%. Larsen et al. (2011) suggests as an explanation that a higher mitochondrial efficacy leads to less VO₂ use. Beetroot seems to have a bigger effect on BP throughout the combination with other compounds than NO₃⁻, as used in this test (McDonagh et al., 2018). During the test measured BP was not significant. Whether systolic BP (t= .085) neither diastolic BP (t= .642). Larsen et al. (2007) tested with a three-day load period of NaNO₃⁻ and a significant difference in BP was shown. The data suggested that the systolic BP is more affected by dietary nitrate ingestion than the diastolic BP. Systolic BP is 3,91% lower with placebo treatment while with esomeprazole just a 2,04% decrease is shown. The diastolic BP seems not to be influenced by the treatment. Esomeprazole leads to a 6,3% decrease while the placebo leads to 6,13% decrease in diastolic BP. It is to assume that the given dose of NO₃⁻ with the duration was maybe not high enough or the loading period was not long enough to show a significant effect. It is as well to consider that BP measurement was too early after the nitrate ingestion. Peak reductions in BP were observed by Bailey et al. (2009) after 2,5 to 3 hours after nitrate ingestion. Natural sources of nitrate in vegetables or their juices have the biggest influence on the BP (McDonagh et al., 2018).

As an important signaling molecule, NO is responsible for many physiological processes (McDonagh et al., 2018). Positive effects are shown to reduce the risk of ischemia or reperfusion issues through protecting tissues from hypertension with vasodilatation (Gladwin et al., 2005; Larsen et al., 2007). As hypertension affects over 1 billion people worldwide, a

nitrate-rich diet is advantageous to minimize the risk of cardiovascular morbidity and mortality by supporting the vasodilatory effect (Chobanian et al., 2003; Wang et al., 2006). This diet would include much vegetables and less processed meat, which can lead to waiver of PPI followed by higher bioactivation of NO. This natural effect on BP, blood flow distribution, muscle contractility, mitochondrial respiration and glucose and calcium homeostasis can at least maintain cardiovascular health throughout time or even enhance it (Bailey et al., 2009; Shen et al., 1994; Stamler & Meissner, 2001; Webb et al., 2008).

A proper and healthy nutrition seems to be the most effective way to prevent taking PPI and increase the bioactivation of dietary nitrate (Lauer et al., 2008). Bouchard-Mercier et al. (2013) suggest a differentiation between a prudent diet- a high in vegetables, fruits, low refined grains, and western diet- processed meat and a high intake of refined products. Those suggestions can be taken to improve or maintain health markers and skip the intake of PPI (McDonagh et al., 2018). This fundamental approach of a higher ingestion of vegetables and nitrate is the least costly way to fight chronic diseases as hypertension or heartburn/ gastroesophageal reflux disease with a proper way of nutrition (Ohlhorst et al., 2013). Taking this nutrition approach by knowing the effects of PPI on the bioactivation of dietary nitrate could be part of future studies.

5. Conclusion

The Purpose of the study was to investigate if PPI (esomeprazole) have an influence on the bioactivation of nitrate due to lowering oxygen cost and BP. After testing, no significant effect is shown. This may occur due to a difficult control of the diet of the subject, the dose and loading phase of NaNO_3^- before the test, differences in sex or the day-time when measuring the participant's BP. Several tendencies are visible, for instance, more utilization of oxygen, less production of carbon dioxide, less ventilation and a lower BP after dietary nitrate intake with the placebo when compared to esomeprazole.

Interestingly, the difference in sex throughout the different metabolisms in female and male lead to different significant outcomes. This data can be used for future studies that a more gender-specific test and a more individual prescription of PPI in dose and frequency may be given, so that results can be more reliable and valid. As well suggestions about the gender specific effects could be make.

In addition, through more movement and more activation of the nitrate-nitrite-nitric-oxide pathway, the bioactivation of NO is bigger but PPI would reduce this effect by affecting the gastric pH. It was not possible to find studies that suggested a special individual diet to prevent the use of PPI to minimize the side effects of chronic exposures of the drug PPI to the body.

In conclusion, I suggest trying to avoid chronic PPI use by staying active and ingesting enough natural dietary nitrate in the form of vegetables to maintain or improve health markers like BP might be a future solution.

More suggestions could have been made if the taken blood samples were analyzed before handing in the study.

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Attachment 1

Litteratursökning/ literature research

Syfte och frågeställningar/Purpose and research question: *Purpose of the study was to investigate if there are difference in measured parameters like VO₂, VCO₂, BP and others to see if there are differences when taking Esomeprazole or a placebo. Research question was to see if PPI (here Esomeprazole) is affecting the bioactivation of dietary nitrate, by influencing the gastric pH, during exercise.*

Vilka sökord har du använt? / Which search word have you used?

Dietary nitrate, Proton Pump Inhibitors, impact PPI nitrate supplementation, Nitric Oxide, Nitrite, bioactivation nitrate, gender/sex specific differences in metabolism/blood, vasodilatation, healthy nutrition

Var har du sökt? / Where did you search?

GIH: s online library, Universität Leipzig online library, PubMed, Ebsco, Google Scholar

Sökningar som gav relevant resultat/ Research that gave relevant results

Ebsco: Dietary nitrate and supplementation bioactivation
PubMed: Proton Pump Inhibitor * effect and nitrate
Google Scholar: gender/sex specific differences *metabolism/blood and vasodilatation and healthy nutrition*

Kommentarer/ Comments

To find fitting material for the introduction was not that hard because the topic is quite good researched and my” handledare” made a lot of the research by himself. It was a little bit more difficult to find the right words for searching the results to proof or consider differences or underline thoughts.

Attachment 2

STUDY INFORMATION

Study on Stomach Acidity and Biological Effects of nitrate

Vegetables have beneficial health effects, and a high intake can reduce the risk of cardiovascular diseases. Recent research shows that nitrate (NO_3^-), which is abundant in certain vegetables may reduce blood pressure in healthy volunteers as well as hypertensive patients. Nitrate is converted in the body to Nitrite (NO_2^-) and further to Nitric Oxide (NO) in the stomach where the stomach acid seems to play an important role. In this study at the Karolinska Institute and Swedish School of Sport and Health Science (GIH) we look at the effects of inhibition of gastric acid production (using proton pump inhibitors) and physiological effects of nitrate.

We are recruiting non-smoking healthy people between 20-65 years of age to participate in this two-day study. You will visit our laboratory for clinical tests and each visit takes about 3.5 hours. It will be at least one week between the visits.

The day before the visit in the lab you are asked to avoid food that are naturally high in nitrate. 18h, 10h, and 2 hours before the visit you will take a tablet that is either a placebo pill or an ulcer medicine of the same type as Losec (the one we use in this study is called Nexium, Esomeprazole 40 mg). Nexium reduces the acidity in the stomach (pH goes up). You will be randomized to start with placebo or Nexium. For 3 hours before the test you will have to avoid eating. You will arrive at the lab at the time agreed on with the test leader and get a venous catheter for blood sampling. We measure blood pressure every 15min with an automatic blood pressure monitor during 1h. After that you will drink nitrate dissolved in a small amount of water. After 90 minutes of rest, you will exercise on a stationary cycle for 20 minutes at a medium effort. The total amount of blood taken in this experiment is 50 ml. For comparison, when donating blood 400-500 ml is given. The last blood sample is taken approximately 11 am and you are free to go home. You will return 7-14 days later to repeat the same procedure.

Your participation is completely voluntary, and you can withdraw from the study at any time without given reason.

If you have questions, please do not hesitate to contact us:

Christopher Eff

E- mail: c.eff@gmx.net

Telephone: +49 151 64996667

Responsible researcher is professor Jon Lundberg

E-Mail: jon.lundberg@ki.se

Telephone: 08 52487952

CONSENT TO PARTICIPATE

Study on Stomach Acidity and Biological Effects of Nitrate and Nitrite

I have read the patient information and I have been orally informed and agree to participate in the study.

I also agree to

handling of my personal data, sensitive data and journal as described in patient information

samples will be stored in a bio bank at Karolinska Institute

samples will be sent for analysis to the Karolinska Hospital/Karolinska Institute

samples will be stored for analysis

samples can be used in other studies after a new approval from the ethics board

samples are stored encoded and that the test and code stored safely and apart

I can withdraw from the study at any time without given reason and my care and treatment will not be affected.

I always have the right to withdraw my consent to saving samples.

Your contact person at GIH is Christopher Eff and Principal Investigator is Dr. Jon Lundberg.

Contact nr: 08-52487952

Printed name and signature of participant

Name	
Date	Signature

The participant has been informed about the study both written and orally.

A copy of the study information and signed consent is given to the participant.

Printed name and signature of investigator

Name	
Date	Signature

Attachment 3

Protokoll Pre-Test

Datum:

Namn:

Pat.-Nr.

Kön:

Ålder:

Nummer (mobil):

Mailadress:

	1. Försöksperson blir informeras om studien och skriver under samtycke																																																												
	2. 3x viloblodtryck: I / mmHg II / mmHg III / mmHg																																																												
	3. Invägning, inmätning och sov timmar Längd: _____ cm Vikt: _____ Kg Sov-timmar _____ h																																																												
	4. Kalibrering och välja syreupptagningsmask																																																												
	5. Pulsband (Vilopuls om känd: _____ bpm)																																																												
	6. Inställning cykel: höjd: styre:																																																												
	7. Max-Test: beroende om storlek och tränings-nivå, kadens/rpm 80																																																												
	<table border="1"><thead><tr><th>Tid</th><th>Watt</th><th>Puls</th><th>Laktat</th><th>BP</th></tr></thead><tbody><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td></td></tr></tbody></table>	Tid	Watt	Puls	Laktat	BP																																																							
Tid	Watt	Puls	Laktat	BP																																																									
	8. FP får tabletter med Losec och placebo och information om intyg																																																												
	9. Information om mat med mindre eller ingen Nitrat. (papper)																																																												
	10. Nästa Försökstillfälle: I II																																																												
	11. Dataanalys																																																												

Attachment 4

Protokoll :a tillfälle

Datum: 2018-

Namn:

Pat.-Nr.

Kön:

Ålder:

Nummer (mobil):

Mailadress:

1. Sätta allt på																								
2. 3x viloblodtryck: I / mmHg II / mmHg III / mmHg Tar sista tablett om inte har gjort innan																								
3. Invägning, inmätning och sov timmar Längd: _____ cm Vikt: _____ Kg Sov-timmar _____ h																								
4. Blodprov:																								
5. Kalibrering och inställning cykel (3 olika sätt)																								
6. Pulsband + mask																								
7. Test del 1 Laktat innan: <table border="1"><tr><td>Laktat:</td><td>80 Watt</td><td>60 RPM</td><td>1,3 Kp</td><td>5minuter</td><td>Puls:</td></tr><tr><td>Laktat:</td><td>80 Watt</td><td>90 RPM</td><td>0,9 Kp</td><td>5minuter</td><td>Puls:</td></tr><tr><td>Laktat:</td><td>120 Watt</td><td>60 RPM</td><td>2,0 Kp</td><td>5minuter</td><td>Puls:</td></tr><tr><td>Laktat:</td><td>120 Watt</td><td>90 RPM</td><td>1,3 Kp</td><td>5minuter</td><td>Puls:</td></tr></table>	Laktat:	80 Watt	60 RPM	1,3 Kp	5minuter	Puls:	Laktat:	80 Watt	90 RPM	0,9 Kp	5minuter	Puls:	Laktat:	120 Watt	60 RPM	2,0 Kp	5minuter	Puls:	Laktat:	120 Watt	90 RPM	1,3 Kp	5minuter	Puls:
Laktat:	80 Watt	60 RPM	1,3 Kp	5minuter	Puls:																			
Laktat:	80 Watt	90 RPM	0,9 Kp	5minuter	Puls:																			
Laktat:	120 Watt	60 RPM	2,0 Kp	5minuter	Puls:																			
Laktat:	120 Watt	90 RPM	1,3 Kp	5minuter	Puls:																			
8. Tar Nitratdos																								
9. Vila 90 minuter																								

10. Test del 2

Laktat innan:

Laktat:	80 Watt	60 RPM	1,3 Kp	5minuter	Puls:
Laktat:	80 Watt	90 RPM	0,9 Kp	5minuter	Puls:
Laktat:	120 Watt	60 RPM	2,0 Kp	5minuter	Puls:
Laktat:	120 Watt	90 RPM	1,3 Kp	5minuter	Puls:

11. Blodprov:

12. Tar med Medicin del 2

Attachment 5

Lista för livsmedel man ska inte äta 24timmar innan man blir testad:

- Spenat (spinach)
- Rödbeta (red beet)
- Gröna sallad (green salad)
- Kål (kale/cabbage)
- Behandlat kött (processed meat)