Unique Protocol ID:	P2016/466 / B406201629930 / NCT03410511
Brief Title:	Propylene Glycol/Glycerol Intake and Cardiorespiratory Function (PGGICF)
Official Title:	Reversibility of Propylene Glycol/Glycerol Intake Effects on Cardiorespiratory Function

1. Background

1.1. State of the art

Electronic cigarettes (e-cigarettes) are increasingly popular worldwide. About 5% of adults in the western world are current users. [1] The liquid (e-liquid) carriers vaporized are composed of propylene glycol (PG) and glycerol (GLY), which transport nicotine and/or flavorings. [2] Regular e-cigarettes users purchase last generation devices delivering high energy level to low coil resistance. [3] High energy settings are used to increase heat and vapor production, [4] to enhance the throat and nicotine hits, [5] whilst nicotine concentration in the e-liquid is strongly reduced through time. E-cigarettes with high output wattage increase the quantity of e-liquid consumed per puff, and produce volatile carbonyls by thermal degradation. [4] Daily exposures to large amounts of high temperature PG/GLY aerosol may present a hazard to health.

1.2. Study of acute vaping effects on cardiorespiratory parameters (2016-*2017*)

We found that acute PG/GLY mix (*50:50*) inhalation: 1) induced a sustained transcutaneous hypoxia without modifying carbon dioxide (CO_2) tension; 2) decreased hemoglobin oxygen saturation, as assessed by fingertip pulse oximeter; 3) injured the lower airway, as reflected by urine and serum club cell protein 16 (CC16) rises, [6] and small airway constriction during lung function tests. [7]

1.3. Study of acute nicotine free vaping effects on arterial O_2 concentration (2017-2018)

At our knowledge, we are the first to demonstrate that acute high wattage PG/GLY vaping induces transcutaneous hypoxia. Transcutaneous hypoxia has been described with tobacco smoking, as a consequence of nicotine-induced vasoconstriction, [9] but effects of a nicotine free PG/GLY liquid vaporization on this parameter were unknown. Tissue oxygen (O_2) and CO_2 tensions reflect the delivery of O_2 and the extraction of CO_2, which are both dependent on respiratory and microcirculatory functions. [10] The decrease in underlying dermal O_2 levels could be explained by: 1) a decrease in arterial O_2 concentration; 2) changes in hemodynamic parameters; and/or 3) microcirculatory endothelial dysfunction. [10] Our current experiments are focusing on acute effects of PG/GLY vaping on arterial O_2 concentration, namely, concentration of hemoglobin, O_2 saturation of hemoglobin and its biological determinants and arterial O_2 partial pressure. [11-2]

1.4. Studying the reversibility of PG/GLY aerosol effects on lungs in regular e-cigarettes users

Our new working hypothesis is that high temperature vaping deposits a hot and moist large amount nanoparticular PG/GLY aerosol deeply in the lungs, [13] resulting in small airways inflammation and constriction,[13] rheological surfactant and mucus properties disturbances,[14] small bronchioles and alveoli collapses, [14] arterial oxygenation impairments, which decrease hemoglobin-oxygen saturation and induce the tissue hypoxia we observed. [10]

In order to verify the new hypotheses aforementioned on PG/GLY vaping induced-lung gas exchanges perturbations and its reversibility, as well as to disentangle respective effects of PG/GLY and nicotine, we will test effects of vaping cessation in regular e-cigarettes users by means of classical cardiorespiratory parameters assessments, [17-19] lung function tests with diffusing capacity analysis and a maximal cycle ergospirometer test. [20] Lastly, quantitative discovery serum proteomics [21] and metabolomics [22] in the serum, urine and exhaled breath

condensate will be performed with the view to highlight biomarkers associated with chronic high wattage vaping exposition.

2. Goals of the research

This research proposal tests the following hypothesis regarding the reversibility of PG/GLY and PG/GLY plus nicotine vaping in regular e-cigarettes users

A. In comparison to pursuit of PG/GLY vaping with or without nicotine, e-cigarette cessation for five days:
   - Increases transcutaneous O_2 tension and O_2-haemoglobin saturation
   - Decreases respiratory rhythm
   - Increases lung diffusing capacities and forced expiratory flows
   - Increases maximal rate of oxygen consumption during cycle ergospirometer test
   - Modifies heart rate variability
   - Decreases arterial stiffness
   - Decreases serum PG/GLY and nicotine concentrations

B. In comparison to PG/GLY vaping with nicotine, e-cigarette cessation for five days or PG/GLY vaping:
   - Increase cutaneous vascular conductance and decrease arterial stiffness
   - Increase acetylcholine and heat mediated vasodilation

C. Each of the three experimental sessions (e-cigarette cessation, PG/GLY vaping and pursuit regular consumptions):
   - Induces specific serum, urine and exhaled breath condensate metabolomics profiles
   - Induces specific serum proteomics profile

This research proposal tests also the following hypothesis regarding acute effects of PG/GLY vaping with and without nicotine in regular e-cigarettes users:

A. In comparison to baseline, PG/GLY vaping without nicotine:
   - Decreases transcutaneous O_2 tension and O_2-haemoglobin saturation
   - Increases respiratory rhythm
   - Increases serum PG/GLY concentrations
   - Induces specific serum, urine and exhaled breath condensate metabolomics profiles

B. In comparison to baseline and PG/GLY without nicotine, PG/GLY vaping with nicotine:
   - Decreases transcutaneous O_2 tension and O_2-haemoglobin saturation
   - Decrease continuous vascular conductance and increase arterial stiffness
   - Increases respiratory rhythm
   - Increases serum PG/GLY and nicotine concentrations
   - Induces specific serum, urine and exhaled breath condensate metabolomics profiles

3. Methodology

3.1. Participants, interventions, periods and randomization

The participants, who are exclusive vapers since at least one years, will undergo three experimental periods in a random order: e-cigarettes cessation for five days (**cessation session**), nicotine free vaping for five days (**nicotine free session**), regular e-cigarettes consumption (**regular consumption session**). Baseline clinical cardiorespiratory parameters will be assessed at the start of each of the 3 experimental sessions. The participants will, then, vape sham-puffs (**cessation session**), nicotine free-puffs (**nicotine free session**) and

nicotine puffs (**regular consumption session**). During acute exposition, we will monitor at each puff any feelings of throat irritation, burn taste and Borg perception scale items (cough, chest tightness, breathlessness, secretions and wheezing). This acute exposition will be followed by clinical cardiorespiratory parameters assessment, lung function tests and maximal cycle ergometer test.

3.2. Measures to ensure strict compliance of the study protocol

All the participants will sign a declaration on honor that they will respect the study protocol. Twelve hours fasting blood samples will be drawn during each experimental session at baseline and 1 hour after acute exposition. All blood samples will undergo serum nicotine, PG and GLY concentration analysis to confirm compliance of the study protocol. [23-26] In case of a protocol violation, the participant will not be allowed to continue the study, and his full dataset will be discarded from the study.

3.2.1 Serum nicotine concentration

At each experimental session, participants will be negative for urine cotinine (urine dipstick – detection threshold of 50 ng/ml – NarcoCheck©) [23] and concentration of serum nicotine should be below 2 ng/ml, a value confirming the status of nicotine free vaping and the lack of significant nicotine exposure. [24,27] Serum nicotine will be measured by means of a mass spectrometer with a jet stream electrospray ion source.

3.2.2 Serum propylene glycol and glycerol

Since serum PG and GLY normal values in regular vapers are unknown, we will take means of PG and GLY serum concentration measured during the **regular consumption session**

and nicotine free session for further comparison to the value obtained during the **cessation session**, in order to confirm the compliance to the study protocol. Serum values of PG and GLY will be assessed using gas chromatography with a flame ionization detector. [25]

3.3 Project plan and various step

The 5h-sessions will take place in the morning at the Hôpital Erasme. During each session, participants will be tested before and after acute exposure at the Cardiology Laboratory (ULB – Pr. Philippe van de Borne) for: 1) noninvasive continuous hemodynamic parameters (Electrocardiogram monitoring (AdInstrument©) and a beat-to-beat hemodynamic monitoring system[17] Finapres Medical Systems©) 2) continuous respiratory rhythm, end-tidal carbon dioxide and blood oxygen saturation (Capnostream©); [28] 3) transcutaneous oxygen and carbon dioxide partial pressures (Perimed©); [10] 4) skin microcirculatory blood flow by means of laser Doppler perfusion monitoring (continuous vascular conductance, heat test, acetylcholine and sodium nipride iontophoresis) (Perimed©); [18] 5) Fractional exhaled carbon monoxide will be assessed at the start of each experimental session using a Smokerlyzer (SineFuma©, Breda, Holland); 6) arterial stiffness indices (Sphygmocor©); [29] 7) exhaled breath condensate (Viasys Ecoscreen©); [19] 8) Urine and 9) venous blood sample. Then, the participants will be directed to the Laboratory of Exercise Physiology, Faculty of Motor Sciences (ULB – Pr. Vitalie Faoro) to perform assessment of: 10) lung function tests plus capillary and membranous lung diffusing capacity as well as maximal ergometer cycle test [20] (details in appendix) (figure 2). After completion of clinical study in all participants, biological samples will undergo proteomics [21] (ULB – Pr. David Communi) and metabolomics [22] (UMons – Pr. Jean-Marie Colet) analysis (details in appendix).

3.3.1 Noninvasive continuous hemodynamic parameters

After 20 minutes of comfortable rest, in the supine position, humeral blood pressure will be determined according to guidelines. [30] Blood pressure measurements will be performed before (baseline) and immediately after vaping sessions or sham-vaping. A cuff will be placed on the middle phalanx of the right middle finger in order to obtain a finger blood pressure waveform with a beat-to-beat hemodynamic monitoring system (Finometer Pro©, FMS, Amsterdam, the Netherlands).[17] This permitted a continuous monitoring of the humeral systolic and diastolic blood pressures throughout all the experimental sessions duration. Pulse rate will be monitored throughout the study by the finometer recordings. In addition a continuous electrocardiogram monitoring will be performed (AdInstrument©).

3.3.2 Continuous respiratory rhythm, end-tidal carbon dioxide and blood oxygen saturation

Continuous, non-invasive, real-time respiratory status monitoring of end-tidal CO2 pressure, O2 saturation in arterial blood, respiration rate, and pulse rate will be assessed with the Capnostream 35 monitor© (Oridion Medical Ltd, Jerusalem, Israël) throughout all the experimental sessions duration.

3.3.3 transcutaneous oxygen and carbon dioxide partial pressures

A PeriFlux system 5000 (Perimed©, Järfälla, Sweden) will explore transcutaneous oxygen (O_2) and carbon dioxide (CO_2) partial pressures by means of a PF 5040 unit and a dual transcutaneous O_2 and CO_2 partial pressures E5280 electrode. After wiping the area with alcohol, a TC 550 fixation ring (Perimed©, Järfälla, Sweden) will be applied to the skin in the medial side of the right arm (lower third and 5 cm distal to the antecubital fossa) at the level of the heart. Four drops of contact liquid (TC 560, Perimed©, Järfälla, Sweden) will be instilled

inside the TC 550 fixation ring. Avoiding superficial vessels and body hair, the electrode will be placed at the same location for each of the two sessions on the basis of multiples pictures taken during the experimental session. Transcutaneous O_2 and CO_2 partial pressures values will be considered stable when variations did not exceed ±2 mmHg within 1 min. Electrode remembraning will be done every two sessions.

3.3.4 skin microcirculatory blood flow by means of laser Doppler perfusion monitoring

3.3.4.1 Skin continuous vascular conductance

Skin continuous microcirculatory blood flow will be assessed using a PeriFlow system 5000, PF 5010/5020 with the thermostatic probe 457 (Perimed©, Järfälla, Sweden) at the level of the right arm at least 5 cm from the PF 5040 with combined transcutaneous O2 and CO2 - E5280 electrode; and mean arterial pressure will be measured throughout the sessions with a beat-to-beat hemodynamic monitoring system (Finometer Pro, FMS©, Amsterdam, the Netherlands) on the right middle finger.[17] Cutaneous vascular conductance will be then calculated as skin continuous microcirculatory blood flow divided by mean arterial pressure.

3.3.4.2 Evaluation of microvascular reactivity to pharmacological stimulation.

“Endothelium-dependent and -independent vasodilatation of the skin microcirculation (measured in perfusion units, PU= 10 mV) will be noninvasively and continuously evaluated by laser Doppler fluxmetry technique in combination with iontophoresis (Periflux 5001 and PeriIont, Perimed, Jarfalla, Sweden) of acetylcholine (ACh), after vaping or sham vaping and iontophoresis of sodium nitroprusside (SNP), minutes after vaping or sham vaping. The laser Doppler flowmetry probes will be constantly heated at throughout the study so as to avoid changes in microvascular blood flow due to local skin temperature variations. Drug-delivery

electrodes (PF 383, Perimed) incorporated into the head of the laser probe (PF 481-1, Perimed), will be carefully placed, by the use of specific double sided skin adhesive patches on the forearms. The drug-delivery electrodes will be filled with 200 µl ACh dissolved in distilled water and will be attached with the laser probe to a standardized site on the right forearm (anterior and proximal part) that had no visible veins. The dispersive electrode will be attached approximately 15 cm away from the electrophoresis chamber, according to the manufacturer's instructions. A protocol of three fixed doses (current intensity-delivery time) will be employed, resulting in an incremental dose±response curve. After measuring the resting flow, three doses of 0.1 ACh dissolved in distilled water will be delivered using a cathodal current (0.1 mA for 80 seconds, with total charges of 8 millicoulombs) at 120-second intervals. The mean values of the resting flow will be considered to be the basal flow values for each patient. Using a new delivery electrode applied on the leff forearm, three doses of a solution of SNP dissolved in distilled water will be delivered using an anodal current (0.1 mA for 80 seconds, with total charges of 8 millicoulombs at 120-second intervals). The curves of the microvascular flow increases induced by SNP and Ach will always have a total recording time of 20 minutes: three times 80-second of iontophoresis at 120-second intervals and 10 minutes to reach the plateau response after the last dose. " [18] (CLINICS 2011;66(4):599-605)

3.3.4.3 Heat test

"Using another laser probe (PF 457, Perimed) at the level of right distal arm (anterior side) that will be positioned at the beginning of the recordings. Maximal skin microvascular vasodilatation will be measured after vaping or sham vaping using prolonged (20 minutes) local heating of the laser probe to 44°C. The use of the area under the blood flow/time curve (AUC) for assessing skin microvascular reactivity using Laser Doppler Perfusion Monitoring is well validated because it represents the global flow response to different physiological and pharmacological stimuli."[33-35] (CLINICS 2011;66(4):599-605)

3.3.5 fractional exhaled carbon dioxide

Fractional exhaled carbon monoxide will be assessed at the start of each experimental session using a Smokerlyzer (SineFuma©, Breda, Holland). Fractional exhaled carbon monoxide should be less than 5 ppm. [36]

3.3.6 arterial stiffness indices

3.3.6.1 Aortic wave reflection assessment

“Using applanation tonometry at the level of left radial pulse, arterial waveforms will be recorded during 8-second with a high-fidelity SPC-301 micromanometer (Millar Instrument©, Texas, USA) by means of a fully automated and validated system (SphygmoCor, Atcor Medical), version 9.0 software (AtCor Medical©, New South Wales, Australia). Three minutes before aortic wave reflection assessments, manual blood pressure measurements (Mercury sphygmomanometer, WelchAllyn©, New York, USA) will be performed according to guidelines[29] in order to calibrate radial pulse wave. Aortic pressure waveforms will be derived from the peripheral radial pressure waves using a validated generalized transfer function. This has been shown to accurately estimate aortic pressure waveforms based on the assumption that mean blood pressure does not change along the arterial tree. In case of decreased aortic compliance or increased peripheral resistance, the reflected wave come back faster to the aorta. This can be assessed by the augmentation index (AIx; 100× augmentation pressure/pulse pressure), representing the pressure boost induced by the return of these reflected waves to the aorta, expressed as a percentage of the pulse pressure. Higher values of AIx indicate an earlier return of the reflected wave to the aorta. Ventricular ejection time determines the aortic pressure waveform. In case of heart rate alterations with ventricular

ejection time modifications, AIx changes may be masked or overestimated. This is why all the AIx presented in the article will be corrected for heart rate (Aix75) according to the linear relationship established by Wilkinson et al,[37] namely, that for every 10-bpm increase in heart rythm, AIx decreases by 4%. These measurements will be made ten minutes before and three times after exposure. " *Clinical and Experimental Pharmacology and Physiology* (2009) **36**, 784–789

3.3.6.2 Pulse wave velocity measurements

"We will assess the carotid-femoral pulse wave velocity (PWV) using a sequential waveform measurements approach at carotid and femoral sites with applanation tonometry (SPC-301 micromanometer) and SphygmoCor software as previously described. [29] The pulse waves time period between the carotid and femoral sites will be assessed with an electrocardiograph-derived R wave as a fixed point. This pulse wave time will be the average of 10 consecutive beats. The difference between the path in cm from the left carotid sampling site to the suprasternal notch, and the path in cm from the left femoral sampling site to the suprasternal notch will be used to define the distance which the pulse wave travels." *Clinical and Experimental Pharmacology and Physiology* (2009) **36**, 784–789

3.3.7 exhaled breath condensate

The participant will close his nose with a nose-clip and approach the mouthpiece. For collecting breathing condensate, the participants will breathe via an elbow piece with connected mouthpiece through a nonrebreathing valve block in which inspiratory and expiratory are separated. During expiration, the breathing air flows through a lamellar condenser which is cooled by the refrigerator circuit. This lamellar condenser is cooled down to -30°C. In this condenser, the breathing condensate is precipitated on the inner wall and drops in the sample collection vessel. The machine which will be used is a ECoScreen

(VIASYS Healthcare GmbH, Hoechberg, Germany). The exhaled breath condensates will be drawn at the start of the experimental and 90 minutes after exposure.

3.3.8 Urine and venous blood samples

Blood will be drawn in the left antecubital vein before and 60 minutes after exposure. Urine specimens will be collected one hour before and 100 minutes after exposure.

3.3.9 Lung function and diffusing capacity evaluation

“In order to detect lung diffusing perturbations at a pulmonary capillary or membrane level induced by vaping, we will assess effects of e-cigarettes cessation on lung diffusing capacities for carbon monoxide (DLCO) and nitric oxide (DLNO) measured with a double nitric oxide/carbon monoxide single breath technique at the Laboratory of Exercise Physiology (ULB – Faculty of Motor Sciences – Pr. Vitalie Faoro). Briefly, the transit of carbon monoxide through the alveolocapillary membrane and into the plasma and intraerythrocytic compartments determines the DLCO, as defined by the Roughton and Forster equation. On the other hand, the DLNO is thought to represent the true membrane diffusing capacity because of its very high affinity for hemoglobin and its independence from pulmonary capillary blood volume. Therefore, the DLNO/DLCO ratio can be used to differentiate thickened alveolocapillary membranes (both DLNO and DLCO are decreased, and the DLNO/DLCO ratio is normal) and decreased perfusion of ventilated alveoli (the DLNO less decreased than the DLCO; therefore, the DLNO/DLCO ratio is elevated).” [20] (*J Appl Physiol (1985)* 2014;116:919-26.)

3.3.10 Maximal ergometer cycle test

“The sessions will consist of an incremental exercise test on a cycle ergometer to determine $\dot{V}_{O_2max}$. Initial workload will be set at 35 W and workload will increase by 15 W with each 1 min stage until volitional fatigue. Breath by breath data will be collected and analysed every 5 s using a metabolic system that will be calibrated with room air and standardized gas. The slope of ventilation relative to carbon dioxide production ($\dot{V}_E/\dot{V}_{CO_2}$) will be calculated with values from rest to maximal exercise. $\dot{V}_{O_2max}$ will be considered to be achieved when two of the following criteria will be met: an increase in oxygen consumption of less than 100 ml min−1 with a further increase in workload, respiratory exchange ratio (RER) greater than 1.1 or achievement of age-predicted maximal heart rate. Pulse oximetry oxygen saturation (S_{pO_2}) will be continuously monitored with a finger pulse oximeter.” Med Sci Sports Exerc. 2017 Oct;49(10):2131-2138.

3.3.11 Serum proteomics analysis

The discovery of differentially expressed protein profiles in e-cigarettes users and its potential reversibility after e-cigarette cessation might be helpful to better understand the physiopathology of tissue hypoxia after vaping and to better characterize the lung inflammation we observed. Serum proteomics will be assessed using recent quantitative sequential window acquisition of all theoretical fragment-ion spectra (SWATH) liquid chromatography/mass spectrometry (ULB – Faculty of Medicine - Pr David Communi). It allows reproducible identification and accurate quantification of thousands of proteins from complex protein mixtures with performance characteristics that approach those of multiple/selected reaction monitoring. Fragment ion spectra from all measurable peptides of a digested proteome are generated through a data-independent acquisition with a much lower ion suppression effect between signals. Moreover, once acquired, the data can be perpetually

analyzed in silico to test new potential biomarkers. [21] The relative quantification of proteins in the serum and the assessment of their potential post-translational modifications between experimental sessions and groups (e-cigarettes users and non-smokers-non-e-cigarettes users) will allow to discover new biomarkers associated with e-cigarettes vaping. Furthermore, the concentrations of these newly discovered biomarkers will be measured in the serum and urine by using dedicated commercial ELISA kits (or by Western blotting followed by immunodetection).

3.3.12 Serum, urine and breath exhaled condensate metabolomics analysis

In order to better understand effects of regular e-cigarettes consumption on human metabolome and to confirm vaping cessation, serum, urine and breath exhaled condensate of our participants will undergo metabolomics analysis. This will be done using nuclear magnetic resonance spectrometry at the Department of Human Biology & Toxicology (University of Mons - Faculty of Medicine – Pr. Jean-Marie Colet) as previously described. [22]

3.3.13 Sample size calculation

The primary objective of this study is to determine whether e-cigarette vaping cessation for five days increases transcutaneous oxygen partial pressure in regular users. Based on our previous researches, we assume that baseline mean transcutaneous oxygen partial pressure will be 80 mm Hg with a standard deviation of 7 mm Hg. To demonstrate that e-cigarette vaping cessation for five days significantly increases transcutaneous oxygen partial pressure, we assume a minimal rise in transcutaneous oxygen partial pressure of 10 mm Hg. We will need 16 patients, with 80% power to detect a group difference of 10 mm Hg in the change of transcutaneous oxygen partial pressure at a two-sided alpha-level of 0.05. We will enroll 21

participants in order to be sure to achieve a sufficient statistical power.

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