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Original Investigation

Effect of the Nicotine Replacement Therapy on Biomarkers of Inflammation, Endothelial Dysfunction, Oxidative Stress, and Lipids in Smokers Who Quit Smoking

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Abstract

Introduction: Our previous study showed major changes in biomarkers on quitting compared to the smoking state. They reflected a decrease in inflammation, endothelial activation, and oxidative stress, as well as an improved lipid profile. Nicotine replacement therapy (NRT) is effective to increase the rate of successful quitting, but healthcare professionals may have concerns to prescribe this first-line smoking cessation treatment because its effect on inflammation and related processes is controversial.

Aims and Methods: The present study assessed the influence of NRT on biomarkers of inflammation, endothelial function, oxidative stress, and lipids, in people who quit smoking. Sixty-five subjects who daily smoke cigarettes were recruited and followed on quitting. Thirty-five quit using NRT and thirty quit without NRT. Biomarkers of inflammation, endothelial function, oxidative stress, and lipids were quantified at baseline when actively smoking and after cessation in the presence of NRT or not.

Results: Changes in biomarkers on quitting did not differ according to the treatment used. No difference was found when comparing participants who were exposed to NRT and those who were not.

Conclusions: These results may indicate that NRT has no effect on inflammation, endothelial function, oxidative stress, and lipids, when used as a medication aid for quitting smoking.

Implications: This study provides new evidence to support the safety profile of NRT products regarding the biomarkers of endothelial function, oxidative stress, inflammation, and lipids.

Introduction

Tobacco use is a leading cause of premature mortality worldwide and is responsible for 8 million deaths each year.[1] Smoking is associated with many health problems, including cardiovascular diseases (CVDs), cancers, diabetes, and respiratory diseases.[2] Chemicals contained in cigarette smoke are responsible for the activation of the endothelium.[3] It leads to a proinflammatory, prothrombotic, and vasoconstrictive state with enhanced oxidative stress, altered expression of adhesion molecules, platelet activation, and impaired vascular tone due to, in part, reduced nitric oxide (NO) bioavailability.[4,5] Inflammation, oxidative stress, and altered lipid metabolism initiate endothelial dysfunction, which is involved in the pathophysiology of many diseases, including CVDs.[6] It has been observed that the risk of disease development was reduced by quitting.[7,8] Our previous study has shown, by the quantification of endothelial dysfunction-related biomarkers, that altered endothelial function observed in people who smoke could be improved by smoking cessation.[9] The levels of interleukin (IL)-6 and soluble intercellular adhesion molecule (sICAM) were found reduced on quitting, reflecting the possible decrease of inflammation and endothelial activation. The concentrations of uric acid and vitamin C, two antioxidants, were higher after the cessation. Finally, lipid profile improvement was observed through the increase of high-density lipoproteins level and the decrease of low-density lipoprotein (LDL) level. No sex-specific difference was visible in the observed biomarkers concentration changes. These changes already occurred in short-term (less than 70 days). All these results emphasizing with a potential rapid reversibility of adverse effects caused by smoking could constitute an additional argument to encourage smoking abstinence.

However, cigarette smoking is an addiction disorder (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[10]) and successful cessation rate without any support is only 4%.[11] Many smoking cessation methods exist to help people quit. The combination of behavioral support and pharmacological treatments seems to

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be the most effective method.[11–13] Among treatments, nicotine replacement therapy (NRT) is widely used and increases rates of quitting by 50%–70%. Nicotine replacement products such as gum, tablet, nasal or oral spray, inhaler, or transdermal patch contain pure nicotine, the major alkaloid responsible for tobacco addiction. Nicotine replacement allows for reducing the severity and duration of withdrawal symptoms.[12,14]

While cigarette smoking is known to lead to endothelial dysfunction, oxidative stress, inflammation, and altered lipid metabolism, the role of nicotine alone in these mechanisms remains still unclear and largely controversial.[4,15,16] For example, during the coronavirus disease (COVID-19) pandemic, some authors raised that nicotine and consequently tobacco may inhibit inflammatory processes and in this way may have a protective action concerning COVID-19.[17,18] Studies in vitro or in experimental animals showed that nicotine increased bioavailability of the vasoconstrictor endothelin-1 (ET-1) and inhibited endothelial production of two vasodilators—NO and prostacyclin (PGI_2). These alterations in endothelial function are associated with oxidative stress, for instance, reactive oxygen species are produced by the uncoupled endothelial NO synthase (eNOS).[16,19] This mechanism promotes an inflammatory state by the expression of chemokines and cytokines due to a reactive oxygen species-damaged endothelium becoming permeable to cells supposed to remain in blood.[19–21] However, these observations are in contradiction with studies indicating an anti-inflammatory effect of nicotine through its binding to the α7 nicotinic acetylcholine receptor (nAChR), which is related to the immune response. Nicotine has been shown to inhibit or down-regulate cytokines production in animal studies.[22,23] Some human studies also suggested the use of nicotine as a therapeutic agent in several diseases and disorders (Parkinson's disease, ulcerative colitis, …).[17,18,22,23] The mechanism involving nicotine as a factor in lipid dysmetabolism is also poorly understood. An animal study showed that nicotine exposure led to enhancing xanthine oxidase activity, resulting in increased triglycerides, triglycerides to high-density lipoproteins ratio, and lipid peroxidation.[24]

The above-described negative effects of nicotine on inflammation, oxidative stress, endothelial function, and lipid metabolism may prevent prescribers from providing NRT for smoking cessation. While our previous study has shown that smoking cessation was associated with changes in biomarkers concentration reflecting the improvement of endothelial function,[9] it seemed to be of interest to determine whether NRT use could affect these beneficial changes. Therefore, this study aimed to compare the changes in biomarkers of inflammation, oxidative stress, endothelial function, and lipids, in people who successfully quit smoking and who were or were not exposed to NRT. A large panel of biomarkers was selected in order to address as fully as possible the different mechanisms implied in these processes: inflammation was studied through the quantification of pro- and anti-inflammatory cytokines, chemokines, and acute-phase protein; oxidative stress was evaluated by prooxidants, molecules or enzymes involved in antioxidant defense, and lipid peroxidation markers; endothelial activation was reflected by adhesion molecules concentration and NO bioavailability; lipid profile was established based on the concentration measurement of various lipids.

Materials and Methods

Subjects

This study follows on from a previous one addressing the concentration changes of endothelial dysfunction-related biomarkers on quitting.[9] Participants are the same. Sixty-five subjects who daily smoke cigarettes, 33 men and 32 women aged 26 to 80 years, were recruited through the tobaccology unit of the Center Hospitalier Universitaire of Catholic University of Louvain (UCL) (Namur, Belgium). All were in good health, without any acute pathology. They were followed during their cessation program. Participants received psychological support and medications for quitting. Thirty-five (21 men and 14 women) used NRT products (gum, transdermal patches, oral sprays, or inhalers), 22 (10 men and 12 women) resorted to varenicline (Champix), and 8 (2 men and 6 women) quit without medication (30 non-NRT-users). The treatment choice was defined by mutual agreement between the tobacco addiction specialist and the participants, depending on contraindications and participants' preferences. A single medication was used, and only a combination of NRT was possible (ie, using short-acting NRT—gum, oral spray, or inhaler—with patches). The compliance with treatment and abstinence was assessed from self-reports during the follow-up tobacco consultation and nicotine and its metabolites measurement. A psychological follow-up was also ensured (craving, behavioral change, etc.). Participants in this study provided informed consent. The project was approved by the Center Hospitalier Universitaire UCL Namur's ethics committee and was led according to the Declaration of Helsinki and the Good Clinical Practice guidelines.[25]

Clinical Protocol

Urine and blood samples from subjects were collected to analyze biological parameters of interest. Urine, one aliquot of heparin whole blood, and one aliquot of EDTA whole blood were immediately frozen at –20°C for later batch analyses. Blood tubes were then centrifuged (15 minutes, 5 ± 3°C, 5950 g, SL16R, Thermo Scientific, USA) to collect serum and heparinized plasma. Some analyses were directly performed on fresh material while aliquots of serum and plasma were frozen at –20°C for batch analyses—chromatographic assays and enzyme-linked immunosorbent assays (ELISA). This protocol was performed twice for all patients: when actively smoking and during their smoking cessation program with or without medication. Participants had to quit smoking for at least 21 days for this second visit. Self-reported tobacco abstinence was confirmed by the analysis of exhaled carbon monoxide (CO) and urinary anatabine and anabasine.[26,27] Compliance with NRT treatment was visible through the presence of nicotine and its metabolites (cotinine and *trans*-3'-hydroxycotinine (3HC)) and the absence of anatabine and anabasine in urine samples.[27]

Quantitative Analyses Instruments

Some parameters were measured directly after blood collection and centrifugation using VITROS 5600 System and VITROS 3600 System from Ortho Clinical Diagnostics (High Wycombe, UK).

The chromatographic systems were products of Waters (Milford, USA): a high-performance liquid chromatography (HPLC) system connected to an ultraviolet (UV) detector (Alliance 2695, detector 2489, Waters) and an ultra-high

performance liquid chromatography system (Acquity UPLC® H-Class). Depending on the analysis, the latter was coupled with photodiode array detector (Acquity PDA), fluorescence detector (Acquity FLR), or mass detector (Acquity QDa). Samples (urine, plasma, or serum) were divided into batches and defrosted on the day of analysis. Systems were calibrated each day of analysis. Data were acquired and processed by Empower 3 (Waters).

Washing steps of ELISA were performed by a microplate strip washer (ELx50, Agilent, Santa Clara, USA) and readings by an absorbance microplate reader (800TS, Agilent). ELISA were carried out on defrosted serum aliquots, allowing batch analyses.

Batch analyses from serum aliquots were also performed on BN ProSpec System (Siemens Healthcare, Saint-Denis Cedex, France).

Biological Parameters Evaluation

Smoking Status

Self-reported abstinence was confirmed by the exhaled CO measurement (Smokerlyzer® monitor, Bedfont Scientific Ltd, Harrietsham, UK). A threshold of 5 ppm was considered compatible with smoking abstinence.[27] Free nicotine, cotinine, 3HC, anatabine, and anabasine were quantified in urine by ultra-high performance liquid chromatography connected to a mass detector by a previously described method.[28]

Oxidative Stress

Uric acid and iron concentrations in serum were determined by colorimetry on VITROS 5600. Ferritin was measured in serum by immunoassay on VITROS 3600. Transferrin was quantified in serum by turbidimetry (reagent pack TFTUR-C00, DiAgam, Ghislenghien, Belgium) on VITROS 5600.

Glutathione peroxidase (GPX) was quantified in defrosted heparin whole blood aliquot by an enzymatic method (kit RANSEL, Randox, Crumlin, UK). Superoxide dismutase (SOD) level was determined in defrosted EDTA whole blood aliquot using a colorimetric activity kit (kit RANSOD, Randox, Crumlin, UK). Both were performed in batches on VITROS 5600.

Chromsystems kits (Munich, Germany) were used to quantify vitamin C in plasma and coenzyme Q10 and β-carotene in serum. Detection was performed by HPLC coupled with UV detector. Vitamin A, vitamin E, and malondialdehyde were extracted from plasma using corresponding Chromsystems kits. Vitamins A and E detection was then achieved through an ultra-high performance liquid chromatography reverse phase column (Acquity UPLC HSS T3 1.8 µm 2.1 × 50 mm, Waters) with a flow rate of 0.6 mL/min and photodiode array detection (scan 210–400 nm). Malondialdehyde was quantified using an ultra-high performance liquid chromatography reverse phase column (Luna Omega 1,6 µm C18 100Å 100X2,1 mm, Phenomenex, Torrance, USA) with a flow rate of 0.3 mL/min and fluorescence detection (excitation wavelength: 515 nm; emission wavelength: 553 nm).

Anti-oxidized LDL (oxLDL) antibodies in serum could be assessed by ELISA kit (Biomedica Medizinprodukte GmbH, Wien, Austria).

Inflammatory Status

ELISA kits from Abcam B.V. (Amsterdam, Netherlands) allowed to measure of monocyte chemoattractant protein 1 (MCP-1) and interleukines (IL) 6, 8, and 10 in serum. Tumor necrosis factor-α was assessed in serum by an ELISA kit from Tecan (IBL International GmbH, Hamburg, Germany). High-sensitivity C-reactive protein (hs-CRP) was quantified in serum by nephelometry using the BN ProSpec.

Endothelial Function

All parameters for the estimation of the endothelial function in this study—Asymmetric dimethylarginine (ADMA), soluble vascular cell adhesion molecule (sVCAM), and soluble intercellular adhesion molecule (sICAM)—were measured in serum by ELISA kits (MT-Diagnostics B.V., Etten-Leur, Netherlands).

Lipid Profile

Serum cholesterol, high-density lipoprotein (high-density lipoproteins), LDL, and triglycerides were assessed by enzymatic methods on VITROS 5600. BN ProSpec was used to quantify lipoprotein (a) (Lp[a]), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB) by nephelometry. Small dense LDL cholesterol was evaluated with an ELISA kit (Cubasio, Houston, USA).

Statistical Analyses

Due to the lack of normality of the data assessed by Shapiro–Wilk test, results are expressed by median with interquartile range into bracket. The comparison of the patient's smoking status when actively smoking and during their smoking cessation program was performed by Wilcoxon signed-rank test. The comparison of the biomarkers modifications when quitting with or without NRT was performed by Wilcoxon Rank Sum Test. Differences were considered significant when $p < .05$. Analyses were carried out using MedCalc (version 20.109, MedCalc Software Ltd, Ostend, Belgium). In case of missing data, the number of samples included in the comparisons was indicated in brackets. No data were deliberately excluded.

Results

Cigarette Abstinence Verification

The patient's smoking status was established from the cigarette consumption self-report, the carbon monoxide (CO) measurement, and the quantification of specific tobacco biomarkers in urine. These data were collected at baseline when actively smoking and after smoking cessation. Data of the 54 subjects in whom the whole set of parameters is available at baseline and after smoking cessation are reported in Table 1. The time of abstinence was variable depending on the patient (median: 70 days, interquartile range: 44.5–104.5 days) but was at least 21 days. Parameters decreased significantly when smoking was stopped. Table 1 shows that nicotine and its metabolites (cotinine and 3HC) were still observed after smoking cessation, since a part of patients received NRT. Absence of cigarette smoking was proved by the low levels of anatabine and anabasine, two alkaloids not derived metabolically from nicotine.

Changes in Tobacco Biomarkers Concentration on Quitting With and Without Nicotine Replacement

The decreases of nicotine, cotinine, and 3HC levels on quitting were significantly lower for patients using nicotine replacement compared to those not using (Table 2). No significant difference was observed in anatabine and anabasine decrease when quitting with NRT or without.

Effect of the NRT on Biomarkers of Inflammation, Endothelial Function, Oxidative Stress, and Lipid Profile on Quitting

Changes in biomarkers of inflammation, endothelial function, oxidative stress, and lipid profile, when smoking was stopped, are shown in Tables 3–6. No significant difference was observed when comparing groups using or not using NRT.

Discussion

The subjects' smoking status was assessed based on self-report and the quantification of tobacco biomarkers. The active smoking status at baseline was confirmed by the exhaled CO level above the reported cutpoint (5 ppm[27]) to distinguish people who smoke and people who do not. Urinary cotinine concentration was also above the cutpoint of 0.03–0.05 µg/mL.[27] People with an active tobacco consumption are reported to have urinary concentrations >0.1 µg/mL for nicotine, >0.4 µg/mL for 3HC,[26,29,30] >0.097 ng/mL for anatabine, and >0.236 ng/mL for anabasine.[31] Cigarette smoking abstinence was then evaluated by the same markers. CO measurement confirmed the cessation of combustible tobacco, the median, and the interquartile range being <5 ppm. Nicotine and its derived metabolites (cotinine and 3HC) were still found in urine (not below the cutpoint values) given the use of NRT for a part of the subjects. However, anatabine and anabasine, not derived metabolically from nicotine, were below the cutpoints. These alkaloids are not found in users of pharmaceutical-grade nicotine, contrary to commercial alternative nicotine product users.[27,32]

As expected, the decreases observed in nicotine, cotinine, and 3HC levels on quitting were lower when using NRT compared to not using, due to the partial nicotine replacement. Since NRT products do not contain any anatabine and anabasine,[27] no significant differences were found in the decrease of these biomarkers when comparing smoking cessation with or without NRT.

Changes in the concentration of biomarkers reflecting inflammation (IL-6, 8, 10, tumor necrosis factor-α, MCP-1, and hs-CRP), endothelial function (ADMA, sVCAM, sICAM), oxidative stress (SOD, GPX, MDA, uric acid, iron, ferritin, transferrin, vitamins A, E, C, coenzyme Q10, β-carotene, and anti-oxLDL), and lipid profile (cholesterol, high-density lipoproteins, LDL, triglycerides, Lp(a), ApoA1, B, sdLDL) on quitting were observed. Our previous study has shown these changes through the follow-up of subjects who entered a smoking cessation program.[9] The biomarkers were quantified at baseline and post-quit, which demonstrated a

Table 1. Patients' Smoking Status at Baseline (Active Smoking) and After Minimum 21 Days of Abstinence (Smoking Cessation)

	Active smoking median (interquartile range) (*n* = 54)	**Smoking cessation median (interquartile range) (*n* = 54)**	***p***
No. of cigarettes per day*	20 (10–25)	0 (0–0)	<.0001
CO (ppm)	17.5 (13.0–25.0)	2.0 (1.0–2.0)	<.0001
Nicotine (µg/mL)	0.612 (0.267–1.006)	0.055 (0.055–0.235)	<.0001
Cotinine (µg/mL)*	1.159 (0.831–1.634)	0.146 (0.010–0.880)	<.0001
3HC (µg/mL)	3.506 (1.651–6.029)	0.413 (0.020–2.271)	<.0001
Anatabine (ng/mL)	5.803 (2.206–11.057)	0.029 (0.029–0.071)	<.0001
Anabasine (ng/mL)	1.866 (0.582–8.062)	0.032 (0.029–0.198)	<.0001

CO = carbon monoxide; 3HC = *trans*-3'-hydroxycotinine. **n* = 64.

Table 2. Tobacco Biomarkers Modification When Stopping Smoking With or Without Nicotine replacement therapy (NRT)

	Changes in NRT-users median (interquartile range) (*n* = 30)	**Changes in non-NRT-users median (interquartile range) (*n* = 24)**	***p***
Nicotine (µg/mL)	−0.189 (−0.563–0.044)	−0.531 (−1.033–−0.179)	.0125
Cotinine (µg/mL)	−0.209 (−0.872–0.164) (*n* = 34)	−1.107 (−1.623–−0.831) (*n* = 30)	<.0001
3HC (µg/mL)	−0.560 (−3.810–0.847)	−4.098 (−5.825–−2.094)	.0005
Anatabine (ng/mL)	−5.123 (−9.385–−1.499)	−6.102 (−12.042–−2.653)	.1858
Anabasine (ng/mL)	−0.947 (−6.591–−0.274)	−2.278 (−8.526–−0.682)	.1055

3HC = *trans*-3'-hydroxycotinine.

Table 3. Concentration Changes in Inflammation Biomarkers on Quitting Smoking With or Without Nicotine replacement therapy (NRT)

	Changes in NRT-users median (interquartile range) (*n* = 35)	**Changes in non-NRT-users median (interquartile range) (*n* = 30)**	*p*
IL-6 (pg/mL)	−0.3 (−1.4–0.9)	−0.7 (−1.5–0.3)	.1174
IL-8 (pg/mL)	−0.9 (−3.1–2.2)	0.5 (−1.4–1.7)	.2925
IL-10 (pg/mL)	0.0 (−0.3–0.9) (*n* = 34)	0.0 (−0.2–0.2) (*n* = 30)	.5540
TNF-α (pg/mL)	0.0 (−0.1–0.0)	0.0 (−0.1–0.0)	.9101
MCP-1 (pg/mL)	−0.7 (−30.7–25.2)	0.4 (−51.8–11.2)	.5895
hs-CRP (mg/L)	−0.16 (−1.04–0.43)	−0.14 (−1.23–0.27)	.6170

IL = interleukine; TNF = tumor necrosis factor; MCP = monocyte chemoattractant protein; hs-CRP = high-sensitivity C-reactive protein.

Table 4. Concentration Changes in Endothelial Function Biomarkers on Quitting Smoking With or Without Nicotine replacement therapy (NRT)

	Changes in NRT-users median (interquartile range) (*n* = 35)	**Changes in non-NRT-users median (interquartile range) (*n* = 30)**	*p*
ADMA (μmol/L)	0.06 (−0.05–0.14)	0.02 (−0.13–0.12)	.3367
sVCAM (ng/mL)	−7.0 (−121.9–60.7)	12.2 (−71.1–96.1)	.2415
sICAM (ng/mL)	−28.0 (−78.3–−1.0)	−10.5 (−68.5–49.0)	.0947

ADMA = asymmetric dimethylarginine; sVCAM = soluble vascular cell adhesion molecule; sICAM = soluble intercellular adhesion molecule.

Table 5. Concentration Changes in Oxidative Stress Biomarkers on Quitting Smoking With or Without Nicotine replacement therapy (NRT)

	Changes in NRT-users median (interquartile range) (*n* = 35)	**Changes in non-NRT-users median (interquartile range) (*n* = 30)**	*p*
SOD (U/mL)	−3.0 (−21.4–28.4) (*n* = 34)	−17.0 (−61.7–14.2) (*n* = 30)	.2500
GPX (U/L)	599 (−3038–2680) (*n* = 18)	−1886 (−2858–427) (*n* = 15)	.1481
MDA (μg/L)	0.68 (−3.21–2.02) (*n* = 19)	−1.09 (−3.70–−0.01) (*n* = 17)	.1406
Uric acid (mg/dL)	0.42 (0.01–0.78) (*n* = 35)	0.31 (−0.26–0.62) (*n* = 29)	.3117
Iron (μg/dL)	3.0 (−25.3–25.8) (*n* = 35)	16.0 (−21.3–36.8) (*n* = 29)	.4420
Ferritin (ng/mL)	0.5 (−18.0–11.8) (*n* = 35)	−1.2 (−10.3–14.0) (*n* = 29)	.7978
Transferrin (g/L)	−0.1 (−0.2–0.1) (*n* = 23)	0.0 (−0.1–0.2) (*n* = 16)	.4075
Vitamin A (mg/L)	0.04 (−0.08–0.12)	−0.01 (−0.04–0.07)	.2925
Vitamin E (mg/L)	−0.60 (−2.86–2.83)	−0.55 (−3.20–1.65)	.7322
Vitamin C (mg/L)	0.92 (−2.33–3.47) (*n* = 32)	0.46 (−1.30–4.34) (*n* = 30)	.7353
Coenzyme Q10 (μg/L)	−19.84 (−206.15–94.28)	−31.08 (−140.26–89.37)	.6170
β-carotene (ng/mL)	−7.18 (−41.34–37.64)	7.47 (−23.67–31.14)	.3237
Anti-oxLDL (U/L)	4.68 (−37.10–79.70) (*n* = 34)	3.55 (−30.30–57.00) (*n* = 30)	.9036

SOD = superoxide dismutase; GPX = glutathione peroxidase; MDA = malondialdehyde; anti-oxLDL = anti-oxidized LDL antibodies.

decrease in inflammation, endothelial activation, and oxidative stress on quitting. An improvement in the lipid profile was also observed. However, these reported changes did not appear to be influenced by NRT exposure. Actually, no significant difference was observed when comparing concentration changes in biomarkers of inflammation, endothelial

Table 6. Concentration Changes in Lipids on Quitting Smoking With or Without Nicotine replacement therapy (NRT)

	Changes in NRT-users median (interquartile range) (n = 35)	Changes in non-NRT-users median (interquartile range) (n = 30)	p
Cholesterol (mg/dL)	−3.0 (−12.3–14.5)	−6.5 (−18.0–12.0)	.4491
HDL (mg/dL)	3.0 (−3.5–6.8)	5.0 (−7.0–12.0)	.5187
LDL (mg/dL)	−8.4 (−17.4–1.3)	−8.0 (−21.8–6.3)	.7521
Triglycerides (mg/dL)	−6.0 (−31.0–38.3)	2.0 (−41.0–47.0)	.7571
Lp(a) (g/L)	0.09 (0.04–0.31) (n = 34)	0.08 (0.03–0.25) (n = 30)	.3335
ApoA1 (g/L)	0.06 (−0.03–0.14)	0.05 (−0.06–0.33)	.6309
ApoB (g/L)	−0.06 (−0.11–0.03)	−0.04 (−0.13–0.05)	.9371
sdLDL (nmol/mL)	−62 (−214–173) (n = 34)	−163 (−495–145) (n = 29)	.2581

HDL = high-density lipoprotein; LDL = low-density lipoprotein; Lp(a) = lipoprotein (a); Apo = apolipoprotein; sdLDL = small dense LDL.

function, oxidative stress, and lipid profile, in NRT-users or non-NRT-users.

To date, there is a lack of data addressing the nicotine exposure effect in humans in real conditions of nicotine use for quitting. Studies are performed in vitro or in animal models and results are sometimes contradictory. Some studies reported that nicotine led to endothelial dysfunction by the deregulation of vasoconstrictors and vasodilators' biodisponibility, which was associated with oxidative stress and inflammation triggers.[19–21] These reported harmful effects could curb doctors to prescribe NRT for quitting while it is known that it increases the cessation success rate.[14] Conversely, other studies claimed an anti-inflammatory effect of nicotine through the cholinergic anti-inflammatory pathway, inducing the suppression of proinflammatory cytokines production.[22,23] This effect of nicotine could protect against infection by different viruses, including influenza A, SARS-CoV-2, etc.[17,22] Authors mention a therapeutic potential of nicotine.[22,23] In this study, no difference in terms of cytokines production was observed when using or not using nicotine with an abstinence of at least 21 days. Similarly, nicotine did not seem to lead to endothelial dysfunction and oxidative stress on quitting. An animal study reported an increase in triglycerides, triglycerides to HDL ratio, and lipid peroxidation due to nicotine exposure[24] but this study does not confirm this hypothesis in humans in cessation.

A limitation of this study is the non-standardization of the duration of cigarette abstinence and NRT use (median: 70 days, interquartile range: 44.5–104.5 days). A hypothesis to explain that no effect of nicotine is observed in biomarkers when stopping is that the use of nicotine could be too short (at least 21 days) to be able to detect any effect. However, this study was performed in real conditions with patients enrolled in a cessation program and samples were collected before a possible relapse. The data obtained here can inform doctors about the short-term use of NRT. Another limitation of this project is the fact that it relates only to the cessation in people who smoke cigarettes and not in people who use other forms of tobacco. The nicotine replacement was also evaluated for admitted NRT products only. Alternative nicotine products were not addressed. Finally, these first results should be confirmed in a larger population.

Conclusion

It is admitted that cigarette smoking leads to endothelial dysfunction, oxidative stress, inflammation, and altered lipid metabolism, but the effect of exposure to nicotine alone is not yet established. This is especially of interest since NRT, containing only nicotine and not the other cigarette constituents, are useful for achieving successful tobacco cessation. Biomarkers of the endothelial function, oxidative stress, inflammation, and lipids, were quantified at baseline and when stopping smoking with or without NRT. Changes in these biomarkers were observed on quitting, but these changes were not significantly different when using or not using NRT. Therefore, NRT products do not seem to affect positively or negatively the studied functions. These data seem to support the safety profile of the NRT products. It could constitute an additional argument for doctors to prescribe NRT to patients enrolled in a smoking cessation program in order to increase their chances of success.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

Declaration of Interests

The authors declare that they have no competing interests. *Financial Disclosures*: None to declare.

Author Contributions

Marie-Lise Colsoul (Conceptualization [Equal], Investigation [Equal], Methodology [Equal], Validation [Equal], Writing – original draft [Equal]), Nicolas Goderniaux (Investigation [Equal], Validation [Equal]), Sabrina Onorati (Investigation [Equal], Validation [Equal]), Stéphanie Dupuis (Investigation [Equal], Validation [Equal]), Jacques Jamart (Conceptualization [Equal], Methodology [Equal], Validation [Equal], Writing – review & editing [Equal]), Dominique Vanpee (Supervision [Equal], Writing – review

& editing [Equal]), Ivan Berlin (Writing – review & editing [Equal]), and Laurence Galanti (Conceptualization [Equal], Supervision [Equal], Validation [Equal], Writing – review & editing [Equal]).

Data Availability

Data will be made available on request.

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