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Effects of emissions from different type of residential heating upon cyclic guanosine monophosphate (cGMP) in blood platelets of residents

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Abstract

We hypothesized that different types of residential heating would be associated with different levels of indoor carbon monoxide (CO) and further that this might result in a differential in the concentration of cyclic 3′:5′ guanosine monophosphate (cGMP) in blood platelets in exposed residents. Individuals, who were recruited from homes using different fuel for heating, donated a venous blood sample in the winter and in the summer. In the winter the median blood platelet cGMP value for the group using liquid propane gas (LPG) was 65% higher than for the group using piped natural gas for heating (p <0.001). Also in the group using LPG, the median concentration of cGMP in the winter was 39% higher than the summer median (p < 0.003). The mean indoor concentrations of CO were measured over a period of 1 week during the winter and were <1 ppm. We conclude that observed differences were associated with emissions from different types of heating but that CO exposure alone is too low to explain these.

Keywords: *Carbon monoxide; cyclic guanosine monophosphate; blood platelets*

Introduction

Are levels of CO in residences a concern?

There are approximately 50 accidental deaths per year in the UK from carbon monoxide (CO) poisoning and over 200 cases of recorded non-fatal injury which can often lead to lasting neurological damage (Chief Medical Officer, Wales 2008). Some 250 000 gas appliances are condemned as unsafe annually and so it may be inferred that a significant number of chronic poisonings remain undetected (Walker & Hay 1999, Kao & Nañagas 2005). The symptoms of chronic low level CO intoxication are non-specific (e.g. fatigue, headache, gastroenteritis) and unlikely to be attributed to exposure (Harper & Croft-Baker 2004). In 2006, a survey of gas appliances in 597 homes in London assigned homes to one of five categories of exposure to CO. Six per cent of homes were assessed as having a 'high or very high' exposure to CO and in these homes 9% of residents reported at least one neurological symptom, i.e. headaches, feeling faint, feeling sick, memory loss, lack of concentration or confusion. Comparison of the high-exposure and least-exposure groups yielded an odds ratio for symptoms of 3.23 (95% confidence interval 1.28–8.15) (Croxford et al. 2008).

The Centre for Disease Control in the USA analysed CO exposure data for the period 2004–2006 taken from the National Electronic Injury Surveillance System – All Injury Program (NEISS-AIP) database. Annually during this period there was an estimated average of 20 636 emergency department visits for non-fatal, unintentional, non-fire-related CO exposures. The analysis showed that the majority of CO exposures, approximately 73%, occurred in the home and it was during the winter months that highest percentage of CO exposures (41.4%) were recorded (Center for Disease Control 2008).

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An investigation in the USA following emergency calls found a high CO level in 3.4% of homes and in these 35% of inhabitants had some of the symptoms potentially attributable to CO toxicity (Jaslow et al. 2001). Inhaled CO binds to haemoglobin in erythrocytes to form carboxyhaemoglobin (COHb) which reduces the oxygen-carrying capacity of haemoglobin. In a survey in the UK, four of 212 elderly patients admitted over the winter with symptoms compatible with CO poisoning had COHb levels over 2% (Croft-Baker et al. 2002). In another survey, eight of 32 elderly patients had COHb levels in excess of 2% of whom two were found to have correctable faults in their heating system (Powis et al. 2002). Decreased exercise tolerance has been observed in patients with coronary artery disease and reproducible exercise-induced angina at COHb concentrations as low as 2.4% (Raub 2000). Baseline estimates of COHb levels expected to be attained by non-smokers exposed to CO can be determined by the Coburn, Forster and Kane model (Coburn et al. 1965). Outdoor air quality standards in the UK (8 ppm as an 8 h running average concentration) and the USA (9 ppm as 8 h running average concentration) have been set on the basis of such effects as well as the epidemiological data as outlined below. The number of surveys of environmental CO levels in residences is small and results are highly dependent on location of monitors and duration of monitoring as well as sample size. In a survey of 821 homes in the UK, CO was monitored in the bedrooms and kitchens using colorimetric diffusion tubes for 2 weeks. The geometric mean level was less than 0.5 ppm and the 95th centile was 2 ppm (Raw et al. 2004). Continuous measurements of CO were made in 44 non-smoking households using an electrochemical sensor and mean concentrations were less than 1 ppm and World Health Organization (WHO) short-term limits (WHO 2002) were not exceeded in any household (Henderson et al 2006). CO was also measured continuously by infrared spectroscopy over a 10-day period in the living rooms of 11 homes and mean concentrations over the period were less than 1.5 ppm (Harrison et al. 2002). In a sample of 270 homes whose occupants satisfied criteria of fuel poverty, (i.e. in receipt of income support and either over 60 years of age or a single parent family) 18% of homes had CO levels above the WHO 8-hour guideline for exposure (Croxford et al. 2006). The effects of poverty were also evident in a survey of 132 homes in New York State with a negative correlation between income and CO levels although mean levels were below 1 ppm (Laquatra et al. 2005). A study of preschool children in Helsinki measured personal exposures to CO and for 4% of the children the WHO 8-hour exposure guideline concentration of 8.6 ppm was exceeded (Alm et al. 2000). The significant contribution of gas heating and cooking to CO concentrations in homes has been generally observed (De Bruin et al. 2004).

Potential effects on health of chronic exposure to low concentrations of CO

Epidemiological data that hospital admissions for congestive heart failure are increased, particularly in the elderly, when ambient CO levels are increased at the 1–10 ppm level lends support a toxicological mechanism (Morris 2000, Burnett et al. 1997, Committee on the Medical Effects of Air Pollutants (COMEAP) 1998). The neuropathological toxicity of CO cannot be explained solely in terms of anoxia and it has been suggested that chronic exposure to low concentrations of CO may have subtle effects on the brain (Townsend &Maynard 2002, Amitai et al. 1998).

The hypoxic effects of severe exposure to CO are well understood but evidence exists for the incorporation of CO with other haemoproteins such as myoglobin and with soluble guanylate cyclase (sGC), inducible nitric oxide synthase and the haem–haemoxygenase (HO) complex which may potentially influence the activity of cellular haemoproteins (Ryter & Otterbein 2004). An indication that a toxic mechanism besides hypoxia might be involved is that there is no clear correlation between COHb levels and the effects on health (Institute for Environment and Health 1998).

The discovery that CO could be involved in biological pathways within cells provides a potential explanation for a toxic mechanism of adverse health effect. The role of molecular gas signal transduction was first recognized when the nitric oxide (NO) molecule was demonstrated to be an endothelial-derived relaxing factor responsible for the regulation of vascular smooth muscle tone (Palmer et al. 1987). The effects of NO on vasodilatation as well as on platelet aggregation and neurotransmission are mediated through the activation of sGC with a subsequent increase in the production of cyclic 3′: 5′ guanosine monophosphate (cGMP) (Bian et al. 2008). cGMP is a cyclic nucleotide produced from guanosine triphosphate (GTP) by the action of both membrane bound and sGCs. cGMP relaxes smooth muscle tissue and it also acts as a common regulator of ion channel conductance, glycogenolysis and cellular apoptosis (Brouard et al. 2000). It is known that CO, like NO, can activate sGC which leads to elevated intracellular cGMP. The importance of NO as a messenger molecule has led to the search for an analogous role for CO. All tissues, including the brain, synthesize haem and the HO system including the constitutive isozyme HO-2 as well as the oxidative stress-inducible protein HO-1 (HSP 32) catalyse the oxidation of haem to produce CO.

A messenger role for CO has been identified for the vascular system (Morita et al. 1995, 1997, Maines 1997, Durante & Schafer 1998). Vascular tone within blood vessels is regulated by CO and a primary mechanism underlying CO cardiotoxicity would appear to relate to

the effects of CO on the HO system and oxidative stress (Gandini et al. 2001, Samutt et al. 1998). It may provide an important adaptive mechanism for moderating the severity of atherosclerosis (Siow et al. 1999) and it has been shown to moderate the severity of vascular injury independent of NO and is a potential therapeutic target for diseases of the vasculature (Duckers et al. 2001).

CO may well act as a neural messenger molecule (Verma et al. 1993, Maines 1993) and probably also functions as a neurotransmitter (Raub & Benignus 2002, Ryter et al. 2002). The parallels between NO and CO suggest that the enzymatic activity of HO-2, like that of nitric oxide synthase (NOS), may be dynamically regulated to provide CO for synaptic activities following neuronal activation (Snyder et al. 1998). Inhalation of CO in rats has been shown to cause delayed impairment of activation of sGC by NO in brain cortex and cerebellum (Hernandez-Viadel et al. 2004). While NO is the major regulator of cGMP in granular cerebellum cell cultures, the endogenous neuronal CO production is high and CO may modulate the NO–cGMP signalling system (Ingi et al. 1996). The mutual regulatory interactions between the CO- and NO-generating systems are complex (Artinian et al. 2001) and may well be affected by exogenous CO. Although NO is much more effective than CO in activating sGC (Stone & Marletta 1994), cells in many tissues either have a constitutively high capacity to generate CO and/or can be induced to do so and some cells are nearly devoid of the ability to generate NO. In some organs, such as the brain, CO-generating capacity greatly exceeds that of NO (Maines 1993).

There is a growing realization that the vasodilatory, anti-inflammatory and immunomodulatory properties of CO demonstrated in several animal models (Ryter & Otterbein 2004) may present potential therapeutic and disease-monitoring applications in clinical practice (Hill-Kapturczak & Agarwal 2006, van Bel et al. 2005, Otterbein et al. 2003). The pathophysiology of CO poisoning is complex (Kao & Nañagas 2006) and the subtle but important role of endogenous CO, which is an active research area in clinical medicine, calls into question the potential effect upon health of chronic low-level exposure to exogenous CO through the same mechanisms.

Biomarkers of effect

The relatively small number of accidental deaths and severe poisonings is important but the question remains whether or not chronic low-level exposure may cause adverse effects on health. Biomarkers of effect which detect functional change in the body resulting from exogenous exposures are finding increased use in molecular epidemiology where a toxic mechanism of adverse health effect is known or hypothesized. There is good evidence, as described above, that physiological roles of CO involve modulation of levels of cGMP. cGMP is therefore a primary candidate for a biomarker of effect on the HO system and the oxidative stress which may underlie cardiac and neurological outcomes. It has been suggested that cGMP could be measured in lymphocytes as a biomarker of CO neurotoxicity (Castoldi et al. 2006).

Temporal dynamics and normal variation of cGMP

NO-mediated activation of cGMP occurs on a timescale of milliseconds to seconds (Feelisch 2007) but little is known about the fine tuning of cGMP elevation and degradation in living cells and tissues due to difficulties in monitoring these temporal dynamics. In platelets and smooth muscle cells an important regulator of cGMP levels is cGMP-specific phosphodiesterase (PDE) 5. In platelets a relatively small increase in PDE5 activity induced by a physiologically occurring NO concentration is sufficient to provide negative feedback and reduce the NO-induced cGMP response for as long as 1 h (Mullershausen 2003). In vascular smooth muscle cells, plateau kinetics arising from higher levels of NO indicate that continuous sGC and cGMP-specific PDE activity determine a steady state level of intracellular cGMP concentrations (Cawley 2007).

cGMP concentrations in blood platelets have only been measured in small groups of healthy individuals and a reference range for the general population is not known. In patients with a mean age of 44 years and with untreated essential hypertension the mean platelet cGMP was 7.0 ± 0.3 pmol 10^{-9} cells (Sala 2009). In 12 healthy male patients aged 28 ± 1.3 years the concentration of cGMP in platelet-rich plasma was 7.5 pmol 10^{-9} platelets (Massuco et al. 2005). However for patients with more severe coronary disease a greater difference in concentration was observed in comparison with healthy individuals. Mean cGMP was 492 ± 201 pmol 10^{-10} platelets in 23 controls, 1089 ± 412 pmol 10^{-10} platelets in 11 patients with unstable angina and 1071 ± 507 pmol 10^{-10} platelets in 12 patients with acute myocardial infarction (Pistono et al. 2002). Severity of coronary artery disease and nitroglycerin medication may influence the levels of cGMP (Nielsen et al. 1999) but in a study of 124 normotensive subjects aged 20–64 years in Austria there was no significant influence of blood pressure, age, heart rate or sex on concentrations of cGMP in plasma. Mean cGMP concentrations in plasma were 2.9 nmol l^{-1} (5th centile 0.4 and 95th centile 5.75 nmol l^{-1}) (Wencker et al. 1989). In a large cross-sectional survey of a general population in Japan urinary cGMP excretion was inversely related to serum total cholesterol (Cui et al. 2005), increased in moderate hypertension but reduced in severe hypertension (Cui et al. 2004) and lowered in the presence of a

high number of metabolic risk factors (Cui et al. 2007). The causal relationships are not known.

We hypothesized that differences in levels of chronic exposure to CO between groups using different types of residential heating would result in a differential in the concentration of cGMP in blood platelets used as a biomarker of effect of exposure.

Methods

Adults over the age of 55 years tend to spend more time indoors than younger adults and so people were recruited in the range of 55–75 years. Further, by restricting the age range the natural population variability in cGMP concentration in blood platelets can be reduced and differential effects between groups using different types of heating more readily observed. Smokers and individuals with a history of either cardiovascular disease, chronic obstructive pulmonary disease or of taking nitrate medication were excluded as these would confound measurements of cGMP attributable to environmental exposure.

Permanent residents were recruited by writing to households in South Wales giving an outline of the study and seeking information on type of fuel used for heating and cooking. A simple questionnaire relating to fuel used for heating and cooking was mailed to 533 homes known to use solid fuel, 219 homes known to use liquid propane gas (LPG) and a random sample of 140 homes in the borough of Neath and Port Talbot. The response rates were 16%, 17% and 70%, respectively, but approximately 50% of all respondents were ineligible with respect to age. Potential participants were informed of the aims of the study and their collaboration sought. Informed written consent was obtained for each participant and ethical approval was granted by the local research ethics committee, in accordance with the Helsinki Declaration of 1975. The proportions of those eligible who were recruited for different fuel groups were: 48/77 for piped natural gas (PNG), 17/19 for LPG, 11/45 for solid fuel and 4/15 for electricity. PNG and LPG heaters were wall mounted with combustion gases vented externally. The numbers of males/females in each fuel group were: PNG (27/21), coal (7/4), electric (2/3), LPG (13/8). During the winter season (October–March) environmental concentrations of CO in the living room were measured every 5 min over a period of 7 days using an electrochemical sensor (City Technology type T3E/F) attached to a tripod and located within the living room. These were calibrated periodically against British Oxygen Company (BOC) certified gas standards and measured down to concentrations less than 1 ppm. During the period that the environmental monitor was in place, the householder was requested to attend the phlebotomy clinic at the local hospital and donate a blood sample. Individuals were recalled to donate a second blood sample 6 months after the initial sample for investigation of seasonal effects. Venous blood samples were collected into pre-prepared vacutainers supplemented with 10 μM Zaprinast, to prevent the breakdown of cGMP, and 10 μM ODQ a sGC inhibitor. The vacutainers were centrifuged at 500***g*** for 10 min and the upper layer of platelet-rich plasma was collected and further centrifuged at 1500***g*** for 10 min. Subsequently, the upper layer of cell-free plasma was carefully removed leaving behind the platelet cell pellets which were stored at -70°C.

The analysis of cGMP was carried out by the Department of Pharmacology within the Welsh Heart Institute. Platelet pellets were resuspended in 1 ml of ice-cold ethanol 65% (v/v) to extract the cGMP from the cells. Following centrifugation at 1500***g*** for 10 min at 4°C the resulting supernatant was removed and evaporated to dryness. The dried sample was resuspended in the appropriate assay buffer and the cGMP content measured by a commercially available radioimmunoassay kit (Amersham Biosciences, Amersham, UK). The pellet of cell debris was dissolved in 1 ml of 1 M sodium hydroxide solution and assayed for protein content using a commercially available kit (BioRad, Hemel Hempstead, UK). The cGMP content of the sample was then normalized to the protein concentration. Results are reported in fmol mg^{-1} protein. Blood samples were also collected into lithium heparin containers for analysis of COHb using an ABL625 series spectrophotometer.

Results

The winter values of cGMP in blood platelets, normalized to protein content, are shown in Table 1 for the different fuel groups. There was an 86% increase in the

Table 1. Concentrations of cyclic 3′:5′ guanosine monophosphate (cGMP) in blood platelets of residents in winter months in homes using different fuels for heating.

Heating fuel type	Number of people	Mean (fmol mg^{-1} protein)	Median (fmol mg^{-1} protein)	Range (fmol mg^{-1} protein)
Gas	48	265.9	220.0	109.2–646.3
Coal	11	321.9	255.3	131.0–704.2
Electricity	4	147.9	149.5	118.8–174.0
LPG	17	544.0	408.3	228–2375.6

LPG, liquid propane gas.

median value of cGMP in participants using LPG to heat their homes compared with those using PNG ($p < 0.001$). There was no statistically significant difference in cGMP between the PNG and LPG fuel groups in the summer due to a marked reduction in the LPG group compared with the winter and only a small reduction in the PNG group: summer median for PNG group 201.5 fmol mg^{-1} and summer median for LPG group 293.8 fmol mg^{-1} as shown in Table 2. The median value of cGMP in the winter for the group using LPG was 39% higher than the value in the summer ($p < 0.003$). The seasonal difference for the group using PNG was only 9% and not statistically significant. There were no statistically significant differences in cGMP concentrations between men and women in either season. The median concentration of cGMP in the group using coal was only 16% higher than that for the group using PNG. The number in the group using coal ($n = 11$) was relatively small and there was no statistically significant difference between this group and the other groups. The difference in cGMP levels between the group using electricity for heating and other groups was not statistically significant as only four such households were included but for this group the mean level was low. In 13 households who used LPG for heating, eight also used LPG for cooking, three used electricity and two used both LPG and electricity. In 37 households who used PNG for heating, 20 used it also for cooking, 11 used electricity and six used both for cooking. Thus the proportions using electricity only (i.e. no emissions of CO or NO) were similar between the PNG and LPG groups and emissions from household cooking appliances are unlikely to have markedly affected the observed differences in cGMP between these two groups. During the winter the mean time, as reported by subjects, for which the heating was switched on was 11 h for the PNG fuel group and 18 h for the LPG fuel group. Occupancy of the home was high with subjects spending 9 h on average in the home over and above time spent sleeping.

Discussion

A considerable and statistically significant difference was observed between concentrations of cGMP in blood platelets in the winter season between groups heating their homes by either PNG or LPG and for the latter group between their winter and summer levels. Whether this observed effect on cGMP between PNG or LPG heating is due to indoor CO exposure in the home is however not clear. Levels of cGMP may be affected by personal exposure to CO which, in addition to the microenvironment of the home, has contributions from the external ambient environment as well as other indoor environments which may contain environmental tobacco smoke (ETS). The homes monitored did not contain a smoker but the general ETS exposure of subjects outside the home was not known. The times that subjects spent in travel in motor vehicles was also not known. Subjects were on average in the home for 9 h in addition to time spent sleeping so whatever their non-home exposures

Table 2. Concentrations of 3′:5′ guanosine monophosphate (cGMP) in blood platelets of residents in summer months in homes using different fuels for heating.

Heating fuel type	Number of people	Mean (fmol mg^{-1} protein)	Median (fmol mg^{-1} protein)	Range (fmol mg^{-1} protein)
Gas	47	224.2	201.5	124.28–595.52
Coal	10	325.4	261.5	57.83–756.7
Electricity	3	343.9	458.6	109.41–463.6
LPG	12	284.2	293.8	68.91–550.48

LPG, liquid propane gas.
There was no statistically significant difference in COHb either between fuel groups or between season: mean COHb 0.18% in the range 0–3.9%. Environmental (Henderson et al. 2006) concentrations of CO in the living room were measured continuously and data logged instantaneously every 5 min over a period of 7 days. The mean environmental concentrations over the period of monitoring were low (<1 ppm) as shown in Table 3 and did not exceed WHO guideline values.

Table 3. Environmental levels (ppm) of carbon monoxide (CO) in winter months in homes using different fuels for heating.

Heating fuel type	Number of households	Number with environmental results	Highest mean[a]	Grand mean[b]	Range of means[c]	Highest peak[d]	Range of highest peak[e]
Gas	37	21	0.88	0.23	0.001–0.88	17.0	0.03–17
Coal	7	5	0.65	0.3	0.003–0.65	6.2	0.08–6.2
Electricity	4	3	0.46	0.33	0.22–0.46	12.0	1.60–12
LPG	13	12	0.98	0.49	0.16–0.98	22.0	0.75–22

[a]Highest mean recorded over the monitored period in any residence; [b]grand mean – the mean of means for each fuel type; [c]range of means for all residences within a particular fuel type; [d]highest peak – highest single peak CO level recorded for each fuel type; [e]range of highest peak for all residences within a particular fuel type.
LPG, liquid propane gas.

they occurred over lesser periods of time. The maximum 8 h running mean of ambient CO concentrations in the general area in which the monitored homes were sited determined from the Automatic Urban Network of Monitoring Stations was 2 ppm but the mean over the winter season was less than 0.5 ppm. It is unlikely that exposures to CO outside of the home are the explanation for the differences observed between those who heated their homes by PNG and those who heated their homes with LPG. Whole body cooling has been shown to trigger an endothelial response resulting in an increase in cGMP levels (Leppert et al. 1997). However, the seasonal temperature difference is unlikely to be the cause of the observed seasonal difference in cGMP measurements for the LPG group. Similarly differences in indoor temperatures are unlikely to be the explanation for the observed differences in cGMP between the PNG and LPG groups in the winter. Subjects had to travel from their homes to a local hospital to donate blood samples. Travel times were not recorded but would have been in the range of half an hour to an hour. The elimination half-life of COHb while breathing air without CO present is 2–6.5 h depending on the initial level of COHb. (WHO 2000). During this travel period if CO exposure was less than in the home then concentrations of cGMP and COHb would have decreased. Although the mean environmental levels of CO were statistically significantly higher for the LPG fuel group (0.49 ppm) than the PNG fuel group (0.23 ppm), the absolute difference would appear to be too small to explain the observed difference observed in cGMP. A possible explanation may lie in the known ability of CO to modulate the activity of endogenous NO (Hartsfield 2002). However an alternative hypothesis may be a hitherto unrecognized direct effect on cGMP from NO generated indoors.

Nitrogen oxides formed in combustion systems are mainly released in the form of NO. The conversion of NO to NO_2 in ambient outdoor air is governed by complex chemical processes in which the chemical coupling of NO_x ($NO + NO_2$) with ozone plays a central role. The Air Quality Expert Group in the UK has considered conversion of NO to NO_2 for vehicular traffic and industrial sources (Department of the Environment, Food & Rural Affairs (Defra) 2004). However the temporal dynamics of the conversion indoors of NO produced by heating appliances to NO_2 may well be different and, further to this, indoors there is a proximity of the person to the emission source. It may well be the case that the effects which we have observed relate more to a differential in NO exposure rather than CO exposure. In this connection a marked effect of atmospheric NO generated by traffic upon the activity of sGC purified from bovine lung has been reported (Friebe et al. 1996).

In conclusion, in our survey the known confounders associated with cGMP are either not present in any group (e.g. nitroglycerin medication) or unlikely to be distributed differently in the groups (e.g. undiagnosed cardiovascular disease). We therefore conclude that the differences observed in cGMP between groups using LPG and PNG in the winter and for the former group between seasons are a real effect associated with emissions from heating type. Indoor levels of CO would seem to be too low in any residence to explain the observed difference in cGMP. We therefore now hypothesize that NO in emissions or possibly the combination of CO and NO may be responsible for effects upon cGMP.

Further research should aim to explore effects in individuals living in indoor microenvironments with a maximum differential in CO and NO emitted from heating appliances. Our data indicate that homes heated by LPG may produce high emissions and the small number of residents who used electricity only for heating and cooking had much lower levels of cGMP as would be expected, although the observed difference did not achieve statistical significance. Measurement of concentrations of CO and NO indoors should be undertaken over extended periods and participants could complete personal activity diaries to record exposures to CO and NO in microenvironments outside the home. It is desirable that serial blood samples are obtained from individuals in their residence.

If more subtle mechanisms of CO intoxication (Townsend & Maynard 2002) exert a chronic effect on health at lower concentrations than those at which the effects due to COHb occur then observed poisoning may represent only the tip of the iceberg with respect to the risks to Public Health from indoor exposure to CO. There is increasing evidence of the diversity of effects of CO in different cell or organ types and genomics and proteomics will in future assist in identifying the molecular targets of CO. Exposure to exogenous CO may interfere and adversely affect these targets either through cGMP-dependent pathways or through other pathways such as the mitogen-activated protein kinases (MAPK). Therefore studies of the interactions of endogenously formed CO and exogenously inhaled CO is an important goal for the future.

The use of cGMP in blood platelets as a biomarker of effect of exposure to exogenous CO and/or NO should be further investigated to answer the question of whether chronic domestic exposure to emissions from indoor heaters may affect the cardiovascular or neurological public health.

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