Partial Inhibition of Nitric Oxide Synthesis In Vivo Does Not Inhibit Glucose Production in Man

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In the liver, paracrine interaction between Kupffer cells and hepatocytes influences glucose metabolism. In vitro in rats, nitric oxide (NO), a paracrine mediator, inhibits several pathways of hepatic glucose production. The role of NO on glucose production has not been studied in vivo in humans. Glucose production was measured during N^G-monomethyl-L-arginine, monoacetate salt (L-NMMA) infusion, an inhibitor of NO synthesis in vivo, in 6 healthy men fasting 23 hours in a saline-controlled crossover study. During L-NMMA infusion, NO output decreased 40% to 50%, peripheral vascular resistance increased approximately 22%, and cardiac output (CO) decreased approximately 14%. The decrease in glucose production was not different between L-NMMA and saline. Glucose concentration, substrate supply, and glucoregulatory hormone concentrations were not different; epinephrine was lower with L-NMMA. A 40% to 50% inhibition of NO synthesis in vivo in humans does not affect glucose production during short-term fasting. The hypothesis that NO is an important modulator of basal glucose production in healthy humans in vivo should therefore be rejected.

IN HEALTHY HUMANS, nonhormonal mechanisms play an important role in the regulation of basal hepatic glucose production. Basal glucose production is maintained at a constant level even in the absence of insulin and glucagon or hepatic vagal activity, and substrates for gluconeogenesis do not influence glucose production as long as their availability is not reduced.[1-5] The mechanism behind this regulatory process is not known. Recent studies show that the primary route by which insulin maintains control over glucose production may be indirect and mediated by free fatty acids (FFA), as was described in the "single gateway hypothesis" by Bergman et al.[6] In the postabsorptive state, under basal conditions, FFA have a clear, but modest, role in the regulation of postabsorptive glucose production, as has been described by Boden et al[7] and Fery et al[8]; inhibition of lipolysis (ie, plasma FFA) with acipimox or nicotinic acid often leaves glucose production unaffected due to autoregulation.[5,7,8] In the regulation of glucose production, intrahepatic factors may play an important role. Experimental data suggest that products of Kupffer cells influence glucose metabolism. Prostaglandins produced by stimulated Kupffer cells regulate glycogenolysis, and in humans, the inhibitor of prostaglandin synthesis indomethacin stimulates glucose production in vivo.[2,9,10] NO is another Kupffer cell product.[11] In vitro studies in rats show that NO strongly inhibits gluconeogenesis, modulates glycogenolysis, and glycogen synthesis.[12,13] In humans, NO has been attributed to effects on insulin sensitivity in hypertension, obesity, and type 2 diabetes mellitus.[14] NO could be a suitable modulator of basal glucose production; NO's short half-life enables a rapid and rapidly reversible effect.[11] Whether NO has a regulatory role in glucose production in healthy humans has not yet been studied. NO is synthesized from L-arginine by various isoforms of the enzyme NO synthase (NOS).[15] Direct measurement of NO production on whole body level is fraught with major problems. In blood, NO has a half-life of seconds due to its binding to hemoglobin, and changes in plasma NO concentration are therefore often deduced from the concentration of nitrate as a stable end product of NO.[11] The plasma nitrate concentration, however, depends not only on NO production, but also on nitrate ingestion and nitrate clearance and is therefore not a very sensitive parameter for whole body NO production.[16-19] The only way to evaluate the potential regulatory role of NO on glucose production during short-term starvation in healthy humans is by inhibiting NO synthesis concomitantly with indirect measurements of NO activity. N^G-monomethyl-L-arginine (L-NMMA), an L-arginine-analogue, is a competitive inhibitor of nitric oxide synthase (NOS) and strongly inhibits NO synthesis in vivo.[11] NO is a major regulator of vascular tone by inducing vascular smooth muscle relaxation; intravenous adminutesistration of L-NMMA to healthy humans raises blood pressure through an increase in systemic vascular resistance.[18]

We hypothesize that if NO is an important modulator of glucose production in vivo, as suggested by the above-mentioned in vitro data, inhibition of NO synthesis may influence basal glucose production. This study evaluated the effect of L-NMMA infusion on glucose production in a saline-controlled crossover study in 6 healthy postabsorptive men. We measured glucose production, concentrations of glucoregulatory hormones and substrates, and direct and indirect parameters of NO production (NO production by the lung, peripheral vascular resistance, and plasma nitrate concentration), between 16 and 23 hours of fasting.

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SUBJECTS AND METHODS

Subjects

Six healthy men (age, 23 ± 0.5 years; weight, 79.1 ± 1.5 kg; height, 1.86 ± 0.01 m; body mass index, 22.9 ± 0.5) participated in the study after written informed consent. All were completely healthy, had not experienced any febrile disease in the month prior to the study, had normal blood pressure, and did not use medication. The study was approved by the Research Committee and the Medical Ethical Committee of the Academic Medical Centre, Amsterdam.

Study Design

Each subject was studied twice, receiving L-NMMA and saline on 1 occasion and saline alone on the other (control study), whereby each subject served as his own control (Fig 1). Both study protocols were separated by at least 4 weeks. For 3 days prior to the study, all volunteers consumed a weight-maintaining diet containing at least 250 g of carbohydrates. The subjects were fasted from 6:00 PM on the day prior to the study until the end of the study. At 7:45 AM, a catheter was placed into an antecubital vein for infusion of stable isotope tracers. Another catheter was inserted retrogradely into a contralateral hand vein kept in a thermoregulated (65°C) plexiglas box for sampling of arterialized venous blood. In both studies, saline was infused as NaCl 0.65% at a rate of 50 mL/h to keep the catheters patent. During both studies, the subjects were confined to bed. At 8:00 AM (t = -2 hours) blood was sampled for determination of background enrichment of [6,6-$^2H_2$]-glucose and then a primed (17.6 μmol/kg), continuous (0.22 μmol/kg/min) infusion of [6,6-$^2H_2$]-glucose (Isotec Inc, Miamisburg, OH) was started and continued until the end of the study at 5:00 PM (t = 7 hours). At t = -20, -15, -10 minutes and t = 0 hour, blood samples for determination of isotopic enrichment of glucose were drawn. At t = 0 hour, blood samples for baseline values of hormones and substrates were also drawn. Immediately after t = 0 hour, a primed (2 mg/kg), continuous (5 mg/kg/h) infusion of L-NMMA (20 mg/mL) (Calbiochem, Novabiochem International, San Diego, CA) was started and continued until 3:00 PM (t = 5 hours).

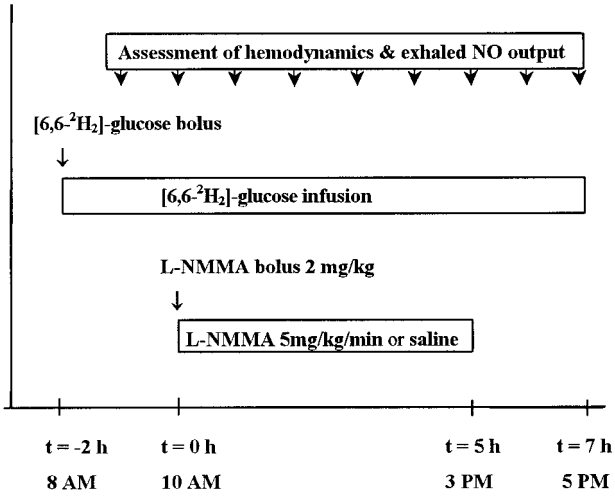

Fig 1. Study design. The bars indicate intravenous (IV) isotope infusion and L-NMMA and/or saline infusion after an initial bolus. t = 0 hour corresponds to baseline measurements and the beginning of the study (16-hour fast). Arrows indicate hourly measurements of hemodynamic parameters and NO output from t = -1 hour to t = 7 hours.

Every 30 minutes between t = 0 hour and the end of the study at 5:00 PM (t = 7 hours), blood samples were drawn for measurement of isotopic enrichment. From t = 0 hour till the end of the study at t = 7 hour blood samples were drawn every hour for measurement of hormone, substrate (FFA, lactate, and alanine), L-NMMA, arginine, and nitrate concentrations. From 3:00 PM (t = 5 hours) until the end of the study at 5:00 PM (t = 7 hours), L-NMMA and arginine concentrations were measured every 15 minutes. The withdrawn blood volume was substituted by at least an equal volume of saline.

NO concentration in exhaled air was measured every hour from t = 0 hour until t = 7 hours after baseline sampling before starting L-NMMA infusion and at t = 24 hours. From 1 hour before the infusion of L-NMMA and/or saline (t = -1 hour) until 2 hours after ending L-NMMA and/or saline infusion (t = 7 hours), continuous noninvasive finger blood pressure was measured every hour for 15 minutes. Changes in cardiac output (CO) and total peripheral resistance were derived from the arterial pressure wave.[20,21]

Assays

All measurements in each individual subject were performed in the same run, and all samples were tested in duplicate. Glucose concentration and enrichment were determined according to Reinauer et al[22] using phenyl-β-D-glucoside as internal standard. The gas chromatography column used was a DB17 capillary column (J & W Scientific, Folsom, CA) on an HP 5890 Series II gas chromatograph coupled to an HP 5989 A model mass spectrometer (Hewlett Packard, Palto Alto, CA). Mass spectra were recorded at m/z 187 for glucose and m/z 189 for 6,6-$^2H_2$-glucose. The internal standard was monitored at m/z 127 and m/z 169. Plasma FFA were determined using the NEFA C kit (code No 994-75409 E) from Wako Chemicals GmgH (Neuss, Germany). Plasma insulin concentration was measured by radioimmunoassay (RIA) (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden; intra-assay coefficient of variation (CV) 3% to 5%, interassay CV 6% to 9%), glucagon was determined by RIA (Linco Research, St Charles, MO; detection limit 15 ng/L, intra-assay CV 3% to 5%, interassay CV 9% to 13%), cortisol was measured using a fluorescence polarization immunoassay (Abbott Laboratories, North Chicago, IL, intra-assay CV 6.4%, interassay CV 9.0%), and catecholamines were measured by in-house high-performance liquid chromatography (HPLC) method. Essentially norepinephrine (inter- and intra-assay CV 13% and 6%, respectively) and epinephrine (inter- and intra-assay CV 14% and 7%, respectively) were selectively isolated by liquid-liquid extraction and derivatized to fluorescent components with 1,2-diphenylethylenediamine. The fluorescent derivatives were separated by reversed phase liquid chromatography and detected by fluorescence detection.[23]

Blood lactate was determined by enzymatic method (Boehringer Mannheim, Mannheim, Germany) on a Cobas Bio Centrifugal Analyzer. L-NMMA, plasma arginine, and alanine concentrations were determined in plasma by amino acid analyzer (Beckman Instruments GmbH, München, Germany; detection limit for L-NMMA is 5 μmol/L), using the modified technique of Moore et al.[24]

The NO concentration was measured in exhaled air during tidal breathing at rest in a sitting position. The nose was clipped and the subjects inhaled NO-free air through a mouthpiece. Expired air was collected via a T-valve in a Douglas bag during a 2-minute procedure. Moreover, respiratory flow and volume data were measured using a ventilated hood (model 2900; computerized energy measurement system, Sensor Medics, Anaheim, CA). NO samples were withdrawn with a vacuum pump through a teflon tube (internal diameter of 4 mm, length 1,700 mm). Measurements were performed from the Douglas bag before starting the L-NMMA infusion for baseline values and then every hour between t = 0 hour and t = 7 hours and at t = 24 hours. NO was measured with a chemiluminescence NO/NOx analyzer CLD

700 AL med (ECO Physics, Durnten, Switzerland), range, 1 to100 parts per billion (ppb). Ambient levels of NO were below 5 ppb. The NO output was calculated as: NO output = NO concentration in exhaled air × minute ventilation/body surface area.[19,25]

Plasma NO_2^- and NO_3^- were determined in deproteinized heparinized plasma. Plasma NO_3^- was converted to NO_2^- using nitrate reductase (Boehringer Mannheim). Total NO2- (ie, plasma NO_2^- + NO_2^- from reduced NO_3^-) and plasma NO_2^- were then determined spectrophotometrically using the Griess reaction.[26] Plasma NO_3^- was calculated as the difference between total NO_2^- and plasma NO_2^- concentration.

Continuous noninvasive blood pressure was measured with a Finapres model 5 (Netherlands Organisation for Applied Scientific Research, Biomedical Instrumentation [BMI-TNO]). Finapres is based on the volume clamp method of Peñáz et al[27] and the Physiocal criteria of Wesseling et al.[28] The Finapres cuff was wrapped around the midphalanx of the index finger of the arm used for the infusion of L-NMMA. The hand with the cuffed finger was carefully positioned at heart level to avoid hydrostatic level disturbances. Finger pressure signal, heart rate, and an event marker were recorded on a thermo-writer (Graphtec; Western Graphtec, Irvine, CA) for direct inspection and stored on a personal computer at a sampling rate of 100 Hz for off-line evaluation. Systolic and diastolic blood pressure was collected from the Finapres signal. Mean arterial blood pressure (MAP) was obtained as the integral of pressure over 1 beat divided by the corresponding beat interval. Heart rate in beats/min was computed from the interbeat interval. CO was computed from the noninvasive arterial pressure wave with the Modelflow method. It uses a nonlinear 3-element model of the aortic input impedance to compute an aortic flow waveform from a peripheral arterial pressure signal.[21] Integrating the computed aortic flow waveform per beat provides left ventricular stroke volume (SV). CO is computed by multiplying SV and instantaneous heart rate. Total peripheral resistance (TPR) is calculated as MAP divided by CO. Changes in CO and TPR were expressed as percentage deviations from baseline (CO [%] and TPR [%]).[31] SV from the noninvasive finger pressure wave in healthy subjects during orthostatic stress tracked a thermodilution-based estimate of SV with a small (3 ± 8 mL) offset.[20] In addition, in critically ill patients, SV from the noninvasive finger pressure wave accurately tracked changes in thermodilution cardiac index for several hours, the overall discrepancy between both measurements was 0.14 $L \cdot min^{-1} \cdot m^{-2}$.[32]

Calculations and Statistics

Because plasma glucose concentrations and tracer/tracee ratios for [6,6-$^2H_2$]-glucose remained constant during each sampling phase of the study, calculations for steady state kinetics were applied and adapted for the use of stable isotopes.[29,33] Glucose clearance was calculated as glucose Ra divided by plasma glucose concentration. For the hemodynamic measurements (MAP, CO, TPR), 4 volunteers served as their own control; for the 2 subjects missing in the saline study, age- and gender-related subjects studied under identical conditions were used. For all the other measurements, all 6 participants served as their own control.

Data are reported as mean ± SEM, unless otherwise stated. Data were analyzed using SAS analysis of repeated measurements (proc mixed; SAS version 6.12, SAS institute, Cary, NC). Because participants served as their own control, the effect of L-NMMA could be calculated by subtracting the differences from baseline values with L-NMMA infusion from the differences from baseline values with placebo. Subsequently, mean difference and standard errors were calculated. Statistical significance was set at $P < .05$.

RESULTS

Clinical Effects of L-NMMA Infusion

Intravenous L-NMMA-infusion was well tolerated and caused no clinical side-effects or laboratory abnormalities in our volunteers (data not shown).

Plasma Concentration of L-NMMA and Arginine

During saline infusion alone, L-NMMA was not detectable in plasma (Fig 2). In the L-NMMA study, plasma L-NMMA levels increased to reach a plateau of around 40 μmol/L after 2 hours (t = 2 hours). After ending the L-NMMA infusion, plasma L-NMMA levels decreased to below the detection limit within 2.5 hours. Baseline arginine concentrations were not different between the L-NMMA-infusion and the control experiment and did not change during or after ending the L-NMMA infusion (t = 0 hour until t = 7 hours: 85 ± 7.2 to 92 ± 6.4 μmol/L [L-NMMA], 84 ± 6.6 to 84 ± 7.1 μmol/L [control]).

Inhibition of NO Synthesis by L-NMMA

Baseline (t = 0 hour) NO output in exhaled air was not different between the L-NMMA and the control experiment (Fig 3). NO output in exhaled air was lower during L-NMMA infusion than during the control experiment ($P = .03$), (t = 0 hour to t = 5 hours: 72 ± 8.1 to 36.8 ± 3.9 nL/min/m^2 [L-NMMA], 73.3 ± 6.9 to 72.7 ± 3.6 nL/min/m^2 [control]). Two hours after ending the L-NMMA infusion (t = 5 hours until t = 7 hours), the NO output in exhaled air had not yet returned to baseline (t = 7 hours: 42.5 ± 8 nL/min/m^2 [L-NMMA] [$P = .02$]). The NO output in exhaled air measured 24 hours after the L-NMMA infusion had started (t = 24 hours) was not different between the L-NMMA and the control group (not shown in figure) (t = 24 hours: 65.9 ± 7.4 nL/min/m^2 [L-NMMA], 90.7 ± 11.3 nL/min/m^2 [control, not significant [NS]).

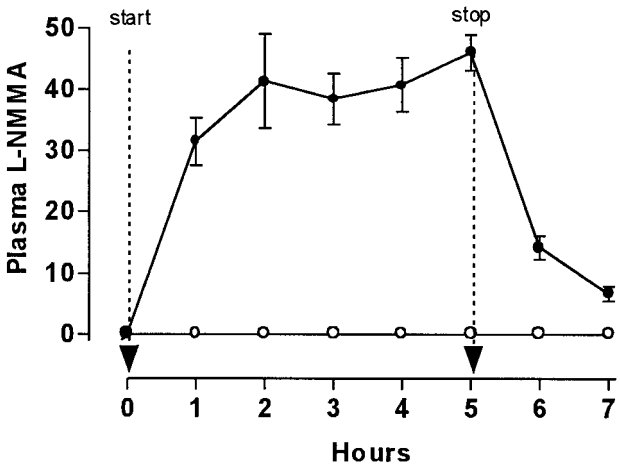

Fig 2. L-NMMA concentration (in μmol/L) in 6 healthy volunteers during and after L-NMMA infusion (●) compared with control (○). "Start" and "stop" arrows demarcate the L-NMMA infusion. Values are means ± SE.

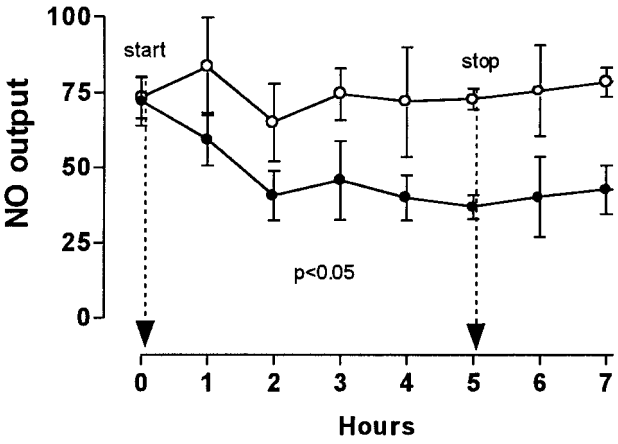

Fig 3. Effect of L-NMMA infusion on NO output (in nL/min/m^2) in 6 healthy volunteers during and after L-NMMA infusion (●) compared with control (○). "Start" and "stop" arrows demarcate the L-NMMA infusion. Values are means ± SE.

Hemodynamic Effects of L-NMMA

Prior to infusion of L-NMMA, absolute values for MAP, CO, and TPR were not different in the L-NMMA and control group (MAP: 72.6 ± 5 mm Hg [L-NMMA] and 71.8 ± 3.3 mm Hg [control]; CO: 3.9 ± 0.15 L/min [L-NMMA] and 4.4 ± 0.35 L/min [control]; TPR: 1,478 ± 127 dyne.s/cm^5 [L-NMMA] and 1,311 ± 66 dyne · s/cm^5 [control]) (Fig 4). TPR % (percentage of baseline) was elevated during L-NMMA infusion compared with control (P <.001), with a maximum difference after 4 hours (Δ 22.5 ± 4.7 %). CO% was lower during L-NMMA infusion (P <.05) with a maximum difference after 3 hours (Δ 14 ± 4.8 %). MAP% was not different between the groups. Both TPR% and CO% had returned to baseline 1 hour after the end of L-NMMA infusion (t = 7 hours, TPR: 99 ± 3.8%, CO: 104 ± 2%).

The Effect of L-NMMA on Nitrate Concentrations

Baseline nitrate concentrations were not different between the L-NMMA-infusion and the control experiment (4.7 ± 8.2 μmol/L [L-NMMA], 41.6 ± 5.1 μmol/L [control]). During both the L-NMMA and the control experiment, the nitrate concentration in plasma decreased linearly between t = 0 hour and t = 7 hours, and there was no difference between the 2 studies (t = 7 hours: 21.7 ± 2. μmol/L [L-NMMA], 20.1 ± 2.8 μmol/L [control]).

Effects of L-NMMA on Glucose Metabolism

Baseline values of plasma glucose concentration and Ra glucose were not different between the L-NMMA-infusion and the control experiment (Fig 5). Between t = 0 hour and t = 5 hours, glucose concentration decreased equally in both groups (5.1 ± 0.07 to 4.9 ± 0.07 mmol/L [L-NMMA] (P < .0001), 5.2 ± 0.08 to 4.9 ± 0.14 mmol/L [control] [P < .0001]); Ra glucose decreased to the same extent [13.5 ± 0.9 to 11 ± 0.9 μmol/kg/min [L-NMMA] (-19%, P < .0001), 12.8 ± 0.8 to 10.6 ± 0.9 μmol/kg/min [control], [-17%, P < .0001]), and glucose clearance decreased to the same extent (2.65 ± 0.19 to 2.26 ± 0.19 mL/kg/min [L-NMMA] [P < .0001], 2.44 ± 0.16 to 2.18 ± 0.17 mL/kg/min [control] [P < .0001]).

Effects of L-NMMA on Substrate Metabolism

Plasma lactate, alanine, and FFA concentrations were not different between the L-NMMA and the control experiment at baseline or during or after the infusion of L-NMMA (Table 1).

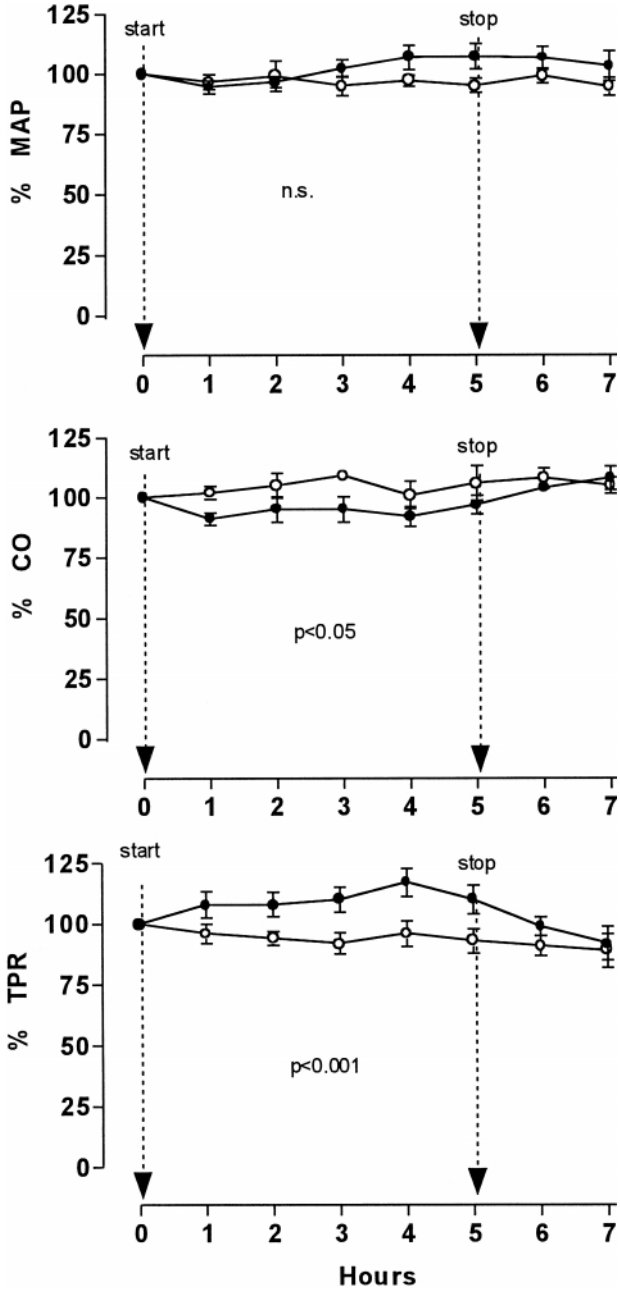

Fig 4. Effect of L-NMMA infusion on hemodynamic parameters. (A) MAP, (B) CO, (C) TPR during and after L-NMMA infusion (●) compared with control (○).Values are expressed as means ± SE of percentual change from baseline. "Start" and "stop" arrows demarcate the L-NMMA infusion.

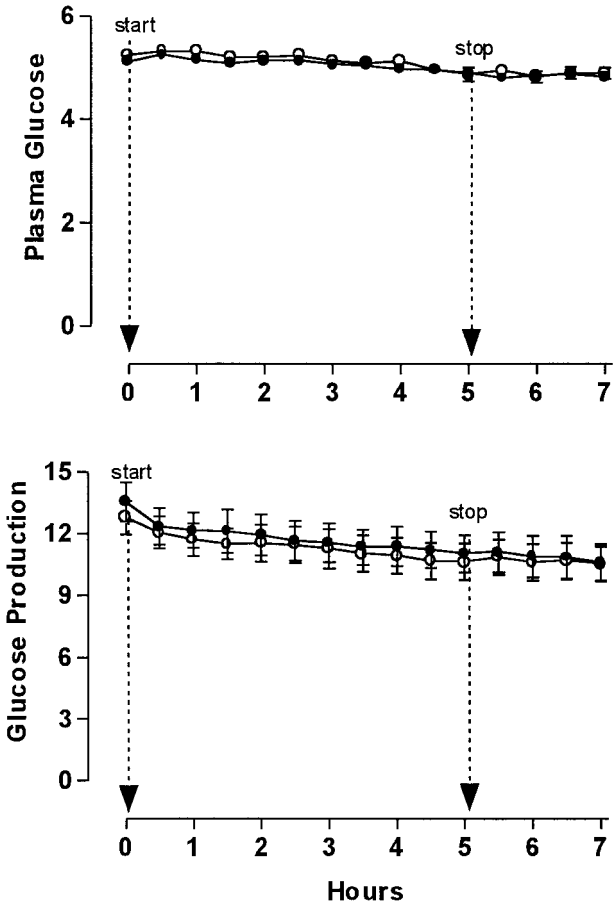

Fig 5. Effect of L-NMMA infusion on glucose concentration in mmol/L (A) and glucose production (Ra) in μmol/kg/min (B) during and after L-NMMA infusion (●) compared with control (○). "Start" and "stop" arrows demarcate the L-NMMA infusion.

Endocrine Effects of L-NMMA

Baseline insulin, glucagon, and cortisol levels were not different between the L-NMMA and control study and did not differ between the L-NMMA and control experiment at any time point (Table 1). Insulin, C-peptide, and cortisol levels decreased significantly in both the L-NMMA and control experiments. Baseline epinephrine and norepinephrine levels were not different between the L-NMMA and control study. During the L-NMMA infusion, epinephrine concentrations were lower than in the control group.

DISCUSSION

In this study, the physiologic effects of NO on postabsorptive glucose production (16- to 23-hour fast) were evaluated by infusion of L-NMMA, a strong inhibitor of NO synthesis in vivo, in healthy humans. We documented that L-NMMA inhibited endogenous NO production as illustrated by a 40% to 50 % reduction of NO output in expired air, and that L-NMMA inhibited endogenous NO production in the cardiovascular system reflected by a 22% increase of TPR and a 14% decrease of CO. Despite this inhibition of NO production in the lungs and cardiovascular system, postabsorptive glucose production decreased as much with L-NMMA as in controls. Therefore, NO is not a major direct or indirect modulator of postabsorptive glucose production in healthy subjects.

The first question that arises is whether the absence of an effect of L-NMMA on glucose production is due to the fact that L-NMMA did not inhibit NO synthesis in the glucose-producing organs. Direct measurement of NO production in the liver and kidney is technically impossible, and the direct measurement of plasma (or whole blood) NO concentration is very difficult in vivo because NO is inactivated within seconds by hemoglobin.[11,19] In vivo, indirect markers of NO production are therefore used for an estimation of local NO production. The most frequently used markers are: (1) plasma nitrate: nitrate is a stable endproduct of NO in vivo, because in the presence of oxyhemoproteins, NO and NO_2^- are rapidly oxidized to NO_3^-[34]; (2) hemodynamic parameters, ie, arterial blood pressure, vascular resistance, and cardiac output; and (3) direct measurement of NO production in exhaled air, as was first described by Gustafson et al.[30] NO concentration in exhaled air reflects endogenous NO production in health and disease, including local production of NO in the respiratory tract and lungs.[19,25] In this study, the NO concentration in exhaled air, systemic vascular resistance, and nitrate concentration in plasma were measured.

Infusion of L-NMMA rapidly caused a 40% to 50% decrease in NO output in exhaled air, which persisted throughout the L-NMMA infusion, a decrease comparable to that described by Kharitonov et al[25] in 67 healthy volunteers undergoing L-NMMA inhalation. The increase in systemic vascular resistance and the decrease in CO measured during L-NMMA infusion in our study are in line with results obtained by others with different doses of L-NMMA.[11,18,35,36] This study showed no effect of L-NMMA on plasma nitrate concentration, despite (in)direct arguments for inhibition of NO production in the lungs and vascular system. The plasma nitrate concentration, however, depends more on endogenous nitrate production, ingestion, and clearance of nitrates than on NO production.[16-19] Although we did not measure dietary intake of our volunteers in this study, we did not find a difference in basal nitrate concentrations between the L-NMMA and control group after 14 hours of fasting. Nitrate clearance is interindividually variable, but the bulk of nitrate is excreted in 24 hours of fasting; studies with L-NMMA are mainly performed in rats and dogs after more than 24 hours of fasting when most of ingested nitrate has already been excreted.[16-18] Studies in humans mainly concern short-lasting experiments in which plasma nitrate concentration is not measured, with the exception of 1 study in 62 healthy subjects, without data on the duration of fasting.[37] Only 1 study in healthy men measured nitrate kinetics after L-NMMA infusion. In that study by Haynes et al,[18] a decrease in nitrate concentration was found after intravenous L-NMMA administration. In our study, baseline nitrate concentrations were twice those in Haynes' study after the same duration of fasting; this suggests a higher contribution of nitrate ingestion to plasma nitrate concentration in our study. It seems likely that the load of ingested nitrate needs to be small to be

Table 1. Plasma Concentrations of Hormones and Precursors

	Group	t = 0 Hour	t = 2 Hours	t = 5 Hours
Insulin (pmol/L)	L	36 ± 3.3	24 ± 3.8	26 ± 2.1*
	C	41 ± 8.3	35 ± 4.7	31 ± 3.4*
C-peptide (nmol/L)	L	0.28 ± 0.05	0.25 ± 0.04	0.22 ± 0.04*
	C	0.41 ± 0.10	0.37 ± 0.07	0.27 ± 0.04*
Glucagon (ng/L)	L	70 ± 12	61 ± 14	70 ± 17
	C	81 ± 24	73 ± 19	70 ± 15
Cortisol (μmol)	L	0.35 ± 0.07	0.30 ± 0.04	0.19 ± 0.03
	C	0.29 ± 0.05	0.29 ± 0.05	0.20 ± 0.02
Epinephrine (nmol/L)	L	0.13 ± 0.03	0.15 ± 0.04	0.17 ± 0.03†
	C	0.16 ± 0.02	0.22 ± 0.05	0.26 ± 0.05
Norepinephrine (nmol/L)	L	1.18 ± 0.27	0.75 ± 0.12	0.52 ± 0.09
	C	0.98 ± 0.16	0.99 ± 0.14	0.98 ± 0.16
FFA (mmol/L)	L	0.66 ± 0.12	0.52 ± 0.11	0.67 ± 0.12
	C	0.43 ± 0.08	0.44 ± 0.11	0.49 ± 0.12
Lactate (mmol/L)	L	0.87 ± 0.11	0.82 ± 0.15	0.88 ± 0.13
	C	0.80 ± 0.08	0.71 ± 0.09	0.56 ± 0.08
Alanine (μmol)	L	244 ± 36	281 ± 39	260 ± 44
	C	302 ± 36	321 ± 57	277 ± 38

NOTE. L rows represent the L-NMMA experiment and C rows the control experiment. L-NMMA infusion starts at t = 0 hour and ends at t = 5 hours.

*$P < .05$ *v* basal (t = 0) values (SAS).

†$P < .01$ L-NMMA *v* control (SAS).

able to detect changes in plasma nitrate concentration derived from NO.

In our study plasma arginine was not affected by L-NMMA. Arginine is the principle substrate for NO synthesis, and Castillo et al[38] have shown that the plasma arginine compartment contributes 54% to whole body NO formation. In the same study, Castillo et al,[38] however, also showed that only 1.2% of plasma arginine turnover is associated with NO formation, which explains why plasma arginine levels did not change with L-NMMA.

To explain the absence of an in vivo effect on glucose production, the theoretical possibility exists that either the L-NMMA concentration was lower in the glucose-producing organs (liver and kidney) than in arterialized plasma or that the influence of L-NMMA on NO production differs site-specifically. The first possibility is unlikely: L-NMMA is water-soluble and hepatocytes will, through fenestration in intrahepatic vessels, be exposed to the same L-NMMA concentrations as those measured peripherally.[39] In vitro, a 40-μmol/L concentration of L-NMMA , comparable to the plasma L-NMMA concentration in our study, caused a 55% inhibition of NO synthesis in macrophages, comparable to the inhibition of NO output measured in exhaled air in our study.[40] From this and from the indisputable effects of L-NMMA seen on NO output and hemodynamic parameters, we deduce that L-NMMA reduced NO production systemically and thus equally so in the glucose-producing organs.

In vitro, pathways of glucose production are strongly inhibited by NO at various levels of glycogenolysis and gluconeogenesis through inhibition of intermediate enzymes, such as phosphoenolpyruvate carboxykinase (PEPck), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glycogen phosphorylase, and glycogen synthase phosphatase.[12,13] Most of these in vitro experiments were performed in rat hepatocytes in the absence of neurohormonal influences. One in vivo study in humans reports on the direct effect of NO synthesis inhibition by L-NMMA on splanchnic glucose production during short-term fasting. In that study, in which a more than 7-fold higher L-NMMA infusion rate was infused than in our study over a short time period (3 mg/kg in 5 minutes), splanchnic glucose output decreased by 26% within 10 minutes, and splanchnic blood flow decreased by 33%.[36] These data are in contradiction with in vitro data and, although not discussed by the investigators, might be explained by the effect of NO on splanchnic blood flow. In our study, we were interested in the role of NO on glucose production during short-term fasting, and a L-NMMA infusion rate was chosen that would inhibit NO synthesis without inducing major hemodynamic changes. Hepatic blood flow was not measured in our study, and we therefore cannot rule out the influence of L-NMMA on splanchnic hemodynamic changes.

Explanations for the difference between in vivo and in vitro results are: (1) species differences, which have been described for another Kupffer cell product, ie, prostaglandins. In humans, prostaglandins inhibit glucose production, whereas in rat hepatocytes, prostaglandins stimulate glycogenolysis.[2,10] (2) NO, in vivo, may be, at best, a weak regulator of glucose production with an effect that needs the absence of glucoregulatory hormones to become overt. (3) Finally, if the production of only a small amount of NO is enough to exert its maximal effect on glucose production, inhibition of NO production needs to be almost complete to induce changes in glucose metabolism. In that case, the remaining 50% NO production found in our study may have sufficed to sustain an effect on glucose production. This seems to be illustrated by the study of Alhborg et al,[41] who showed that excessive inhibition of NO synthesis had an effect

(directly or indirectly) on glucose output by the liver. From the above mentioned, we deduce that NO can only be a weak regulator of glucose production in the physiologic state, and that its effect may only become overt under extreme conditions of overproduction or inhibition, or when hormonal counterregulation is not effective on glucose production. This conclusion only relates to the regulation of glucose production in healthy subjects. It is possible that NO has a more important role in diseases in which glucose production is disturbed, as it is in type 2 diabetes. This is a real possibility, as we have shown for other mediators, that their effect is different in healthy subjects and in diabetic patients.[42]

Plasma epinephrine concentrations decreased during L-NMMA infusion and returned to control values shortly after the end of the L-NMMA infusion. This is the first study to describe a decrease in plasma epinephrine concentration during L-NMMA infusion in vivo in humans. The in vitro studies performed up to date on the regulating role of NO in adrenal catecholamine secretion are controversial.[43] The question is whether, with epinephrine concentrations in the L-NMMA experiment similar to those in controls, glucose production would have been higher. The plasma epinephrine concentration, however, required to increase glucose production is much higher than the epinephrine concentrations found in either of our groups during the study. This is inconsistent with a role for epinephrine in our study.[44]

In conclusion, the results of this study show that a 40% to 50% inhibition of NO synthesis during short-term fasting does not influence overall glucose production in vivo in humans. In vitro, NO was shown to inhibit several pathways of glucose metabolism. In vivo, glucose production is a tightly controlled system of fluxes through different pathways, and stimulation of 1 pathway leads to inhibition of another to keep net glucose production constant.[5] The results of this study, therefore, do not exclude an influence of NO on specific pathways of glucose production in vivo.

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