Corrections

PHARMACOLOGY. For the article “Beneficial effects of antioxidants and L-arginine on oxidation-sensitive gene expression and endothelial NO synthase activity at sites of disturbed shear stress,” by Filomena de Nigris, Lilach O. Lerman, Sharon Williams Ignarro, Giacomo Sica, Amir Lerman, Wulf Palinski, Louis J. Ignarro, and Claudio Napoli, which appeared in issue 3, February 4, 2003, of Proc. Natl. Acad. Sci. USA (100, 1420–1425; first published January 13, 2003; 10.1073/pnas.0237367100), the authors should have noted that Louis J. Ignarro developed and markets Niteworks, a dietary supplement of L-arginine, L-citrulline, vitamin E, and vitamin C. Dr. Ignarro is also a member of the Scientific Advisory Board of Herbalife, the distributor of Niteworks.

PHARMACOLOGY. For the article “Long-term combined beneficial effects of physical training and metabolic treatment on atherosclerosis in hypercholesterolemic mice,” by Claudio Napoli, Sharon Williams-Ignarro, Filomena de Nigris, Lilach O. Lerman, Loredana Rossi, Carmen Guarino, Gelsomina Mansueto, Francesco Di Tuoro, Orlando Pignalosa, Gaetano De Rosa, Vincenzo Sica, and Louis J. Ignarro, which appeared in issue 23, June 8, 2004, of Proc. Natl. Acad. Sci. USA (101, 8797–8802; first published May 28, 2004; 10.1073/pnas.0402734101), the authors should have noted that Louis J. Ignarro developed and markets Niteworks, a dietary supplement of L-arginine, L-citrulline, vitamin E, and vitamin C. Dr. Ignarro is also a member of the Scientific Advisory Board of Herbalife, the distributor of Niteworks.

COMMENTARY. For the article “Flow, NO, and atherogenesis,” by John P. Cooke, which appeared in issue 3, February 4, 2003, of Proc. Natl. Acad. Sci. USA (100, 768–770; first published January 27, 2003; 10.1073/pnas.0430082100), the author should have noted that this work was supported by the National Heart, Lung, and Blood Institute (Grants R01 HL-63685, R01 HL-75774, R01 AT/HL00204, P01 AG18784, and P01 AI50153). Dr. Cooke is the inventor of patents owned by Stanford University for asymmetrical dimethylarginine (ADMA) assays and for the use of L-arginine in cardiovascular disease, for which he receives royalties. Dr. Cooke is a consultant for United Therapeutics.
Flow, NO, and atherogenesis

John P. Cooke*

Program in Vascular Medicine and Biology, Stanford University School of Medicine, Stanford, CA 94305

Atherosclerosis is the major cause of death and disability in the United States, Europe, and much of Asia. The lesions of atherosclerosis have a nonuniform distribution in the vasculature and are prone to develop at bends, branches, and bifurcations of the aorta and its conduit arteries. At these sites in the vasculature laminar flow is disturbed by recirculation, which resembles an eddy at a river branch. Here the endothelium of the blood vessel is exposed to reduced and oscillating flow, a hemodynamic condition that produces physicochemical and biological alterations predisposing to atherogenesis (Fig. 1). Recirculation causes an increase in particle residence time, so that lipoproteins and monocytes (the anlagen of atherosclerosis) have greater contact with the endothelium in these regions (1). The endothelium itself is morphologically altered. In relatively straight segments of a conduit vessel, endothelial cells are regularly aligned, each cell with its longitudinal axis oriented in the direction of flow. By contrast, in areas of disturbed flow, the usual orientation of endothelial cells is lost, and the cells become more polygonal in appearance. Intriguingly, endothelial cells at branch points appear to age faster. Endothelial cells exposed to disturbed flow in vitro turn over more rapidly (2). In human iliac arteries, endothelial cells at the bifurcation have shorter telomeres, consistent with a focal acceleration of senescence (3). Senescent human endothelial cells produce less nitric oxide (NO), generate more superoxide anion (O$_2^-$), and are more adhesive for monocytes, effects that can be reversed by transfection with the gene encoding telomerase (4).

Laminar flow in a straight segment of the conduit vessel exposes the endothelium to the tractive force of shear stress. Shear stress in a physiological range of 10–40 dynes/cm$^2$ (1 dyne = 10 $\mu$N) activates signaling pathways that induce endothelial elaboration of a number of vasoactive factors such as NO, prostacyclin, tissue plasminogen activator, and transforming growth factor $\beta$ (5–8). Nitric oxide is paradigmatic of these factors that promote vasodilatation, inhibit adherence of circulating blood elements, and suppress the proliferation and migration of vascular smooth muscle cells (9). In addition to triggering the immediate release of NO, laminar shear stress increases the expression of NO synthase (NOS) (10), and that of superoxide dismutase (which reduces oxidative degradation of NO; ref. 11). Thus, in a physiological range, laminar shear stress maintains vascular homeostasis. By contrast, disturbed flow (causing low and oscillating shear stress) inhibits the release of these factors. At the branches of coronary arteries in humans, endothelium-dependent vasodilatation is impaired (12). Similarly, when cultured endothelial cells are exposed to disturbed flow, NOS expression is not up-regulated as when endothelial cells are exposed to laminar flow (13). This hemodynamic alteration of endothelial biology predisposes to processes involved in atherogenesis. This theme is further explored by de Nigris et al. (14) in studies that document the interaction of shear stress, NOS, and proatherogenic transcriptional factors. They find that in the aorta of the hypercholesterolemic low-density lipoprotein receptor-deficient mouse, the expression of NOS is down-regulated, whereas expression of the proatherogenic transcriptional factors Elk-1 and p-CREB is increased. This alteration in protein expression is particularly evident at lesion-prone sites in the aorta, i.e., areas of disturbed flow. The reduction in NOS expression was reversed by administration of L-arginine or the antioxidant vitamins C and E. There was a synergistic interaction of L-arginine and the antioxidant vitamins to increase NOS expression and reduce Elk-1 and p-CREB. Moreover, the ad-
ministration of L-arginine or the anti-
oxidant vitamins suppressed lesion
formation, again with a synergistic
interaction. They observed a similar
interaction between antioxidant vita-
mins and L-arginine on the expression
of NOS, Elk-1, and p-CREB in cul-
tured endothelial cells exposed to vari-
able levels of shear stress by using a
cone-plate viscometer.

The balance between vascular gener-
ation of two free radicals, NO and O2·
−, is a major determinant of endothelial
adhesiveness and lesion formation.
Cultured endothelial cells exposed to
physiological laminar flow generate
more NO and less superoxide anion;
consequently, activation of the tran-
scriptional protein NF-κB is attenu-
ated, expression of the endothelial ad-
hesion molecule VCAM-1 is reduced,
and endothelial adhesiveness for mon-
cytes is diminished (15). As confirmed
by de Nigris et al. (14), the balance
between vascular NO and O2·− can also
be shifted by administration of antioxi-
dants or the NO precursor L-arginine.
In cell culture and animal models of
hypercholesterolemia and atheroscle-
rosis, L-arginine becomes rate limiting
for NO production (16, 17). This may
be caused by increased tissue or
plasma levels of the endogenous inhibi-
tor of NOS, asymmetric dimethylargi-
ine; increased expression of vascular
arginase; reduced L-arginine transport;
and/or increased local utilization of
L-arginine by immune or vascular cells
expressing inducible NOS (18–21). In
the setting of reduced availability of
L-arginine, NOS transfers electrons to
oxygen to create superoxide anion
(22). Superoxide anion generated by
NOS, NADPH oxidase, mitochondrial
oxida
des, or other enzymes oxidizes
to the highly reactive and cytotoxic
free radical peroxynitrite anion (23).
Furthermore, oxidative stress attenu-
ates the expression of endothelial
NOS, in part by reducing mRNA stabi-
ility (24). Finally, oxidant-responsive
genuses such as VCAM-1 or MCP-1 are
induced, increasing monocyte adhesion,
infiltration, and lesion formation (15,
25). Under these conditions, L-arginine
and/or antioxidants can reverse the
pathobiology induced by oxidative stress
(18, 26).

Whether interventions to enhance
endothelial NOS and/or reduce oxida-
tive stress will be clinically useful re-
main to be determined. L-arginine
supplementation has been shown to
improve endothelial function, enhance
coronary blood flow, reduce angina,
and improve exercise tolerance in pa-
tients with coronary artery disease
(CAD; refs. 27 and 28); however, there
are no data regarding its effects on
progression of CAD or cardiovascular
mortality. Although antioxidants C and
E can improve endothelial function in
humans with CAD or with risk factors
for CAD (29), large randomized clini-
cal trials with antioxidant vitamin ther-
apy have been for the most part disap-
pointingly negative (30). In fact, high
doses of antioxidant vitamins blunt the
beneficial effects of statins to increase
high-density lipoprotein cholesterol
and reduce progression of CAD (31).
By contrast, statins or angiotensin-con-
verting enzyme inhibitors reduce the
progression of CAD and total mortal-
ity (32, 33). Some of the benefit of
these agents has been ascribed to their
effect of improving endothelial func-
tion (e.g., uncoupling NOS activity and
expression, reducing the generation of
O2·−), but this remains conjecture. How-
ever, endothelial dysfunction (as mani-
ifested by reduced vasodilatation to
endothelial agonists, or by increased
plasma levels of the NOS inhibitor
ADMA) has been shown in multiple
studies to be an independent pre-
dictor of the severity and sequelae
cardiovascular disease (34–39). Thus
endothelial-targeted diagnostics
and therapy are likely to be important
tools in the future management of
cardiovascular diseases.

**Endothelial
dysfunction is an
independent predictor of the severity
of cardiovascular disease.**

1. Glagov, S., Zarins, C., Giddens, D. P. & Ku,
1031.
2. Davies, P. F., Remuzzi, A., Gordon, E. J., Dewey,
Natl. Acad. Sci. USA 83, 2114–2117.
Sci. USA 92, 11190–11194.
4. Matsushita, H., Chang, E., Glassford, A. J.,
Res. 89, 793–798.
1663–1671.
8. Cimo, M., Cooke, J. P., Dzau, V. J. & Gibbons,
Med. 48, 489–509.
10. Uematsu, M., Ohara, Y., Navas, J. P., Nishika,
Murphy, T. J., Alexander, R. W., Nerem, R. M.
& Harrison, D. G. (1995) Am. J. Physiol. 269,
C1371–C1381.
11. Topper, J. N., Cai, J., Falb, D. & Gimbrone, M. A.,
10422.
12. McLenachan, J. M., Vita, J., Fish, D. R., Treasure,
Circulation 82, 1169–1173.