Decreased Blood Flow But Unaltered Insulin Sensitivity of Glucose Uptake in Skeletal Muscle of Chronic Smokers

E.M. Rönnemaa, T. Rönnemaa, T. Utriainen, M. Raitakari, H. Laine, T. Takala, O.-P. Pitkänen, O. Kirvelä, J. Knuuti, and P. Nuutila

Chronic cigarette smoking is associated with dysfunction of the vascular endothelium. Smokers have also been shown to be insulin-resistant, at least in some studies. Since insulin-induced vasodilation is dependent on endothelial cell nitric oxide (NO) synthesis, we tested the hypothesis that decreased skeletal muscle blood flow causes insulin resistance in smokers. We studied 37 young normotensive normolipidemic nondiabetic men, of which 14 were smokers and 23 lifelong nonsmokers. The groups were similar with respect to age, body mass index (BMI), and maximal oxygen uptake ($\dot{V}O_2$max). Basal and insulin-stimulated femoral muscle blood flow was measured using [^{15}O]H_2O and insulin-stimulated muscle glucose uptake using [^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and positron emission tomography (PET). Whole-body glucose uptake was measured using the hyperinsulinemic (insulin infusion 5 mU/kg · min)-euglycemic clamp technique. In the basal state, muscle blood flow was 51% lower in smokers (17 ± 3 mL/kg muscle · min) versus nonsmokers (35 ± 17 mL/kg · min, $P < .0001$). Insulin increased muscle blood flow comparably in both groups; the mean rate of insulin-stimulated blood flow was 30 ± 10 and 55 ± 38 mL/kg · min ($P = .049$), respectively. Whole-body and skeletal muscle glucose uptake were similar in both groups during insulin infusion. We conclude that muscle blood flow is lower in chronic smokers compared with nonsmokers under both fasting and hyperinsulinemic conditions. The insulin-induced increase in muscle blood flow and insulin-stimulated glucose uptake appear normal, suggesting that the vasodilatory and metabolic effects of insulin are intact in smokers and the reduced muscle blood flow per se does not cause insulin resistance in these subjects.

CIGARETTE SMOKING has been shown consistently to be a major risk factor for atherosclerosis, and it is strongly associated with coronary, cerebral, and peripheral vascular disease.[1] Tobacco smoke contains numerous toxic substances, but it is still incompletely understood as to how smoking elicits harmful vascular effects. Smoking is acutely followed by several hemodynamic changes such as an increase in heart rate,[2-5] blood pressure,[3-5] and coronary vascular resistance[6] and a decrease in compliance of the brachial artery.[4] Total forearm blood flow has been reported to decrease,[3] to remain unchanged,[4,5] or even to increase[7] after smoking. These changes are accompanied by increased plasma catecholamine concentrations.[2,5] It is presently unknown whether the acute hemodynamic effects of smoking, when repeated many times per day, induce permanent hemodynamic changes in smokers.

Increased fasting serum insulin and triglycerides and decreased high-density lipoprotein (HDL) cholesterol, indirect markers of insulin resistance and independent risk factors for cardiovascular disease, have characterized smokers in several studies.[8-10] However, the direct evidence linking smoking with the insulin resistance of glucose uptake is controversial. Impaired insulin-stimulated glucose uptake has been found in some studies,[8,11] but no such association has been found in others.[9,12] In previous studies, smokers and nonsmokers generally have been matched for age, body mass index (BMI), and adiposity but, without exception, not for physical fitness. This is unfortunate, since the latter parameter is an important determinant of insulin sensitivity in normal subjects[12,13] and may confound the comparison of any two groups independently of smoking status.

Endothelial dysfunction, an early marker of atherosclerosis, also has been found to characterize chronic smokers in some[14-19] but not all[20,21] studies. At least large doses of insulin[22,23] have a vasodilatory effect that is localized to skeletal muscle[24] and mediated by endothelial cell nitric oxide (NO) synthesis.[25,26] However, since there are no studies examining limb or muscle blood flow in chronic smokers during hyperinsulinemic conditions, it is also unknown whether the vasodilatory action of insulin, ie, the blood flow response to insulin, is impaired in these subjects. If this is the case, it may contribute to the proposed resistance to the effect of insulin to stimulate muscle glucose uptake in smokers.

To examine whether smoking is associated with the insulin resistance of glucose uptake in skeletal muscle independently of physical fitness, and whether smoking is associated with the impaired muscle blood flow response to insulin, we measured muscle blood flow and glucose uptake under fasting and normoglycemic-hyperinsulinemic conditions in a group of chronic smokers and a group of nonsmokers matched for age, BMI, and maximal oxygen uptake ($\dot{V}O_2$max).

SUBJECTS AND METHODS

Subjects

A total of 37 normal male subjects were studied; 14 were smokers and 23 were nonsmokers. The smokers regularly smoked at least five cigarettes per day for at least 3 months preceding the study. Nonsmokers had never smoked. Ex-smokers and habitual smokers were not included in the study. The smokers and nonsmokers were similar with respect to age, BMI, and maximal oxygen uptake ($\dot{V}O_2$max). Insulin-stimulated muscle blood flow and glucose uptake were measured in subgroups of smokers (n = 8) and nonsmokers (n = 12). These subgroups of smokers and nonsmokers were also similar with respect to age, BMI, and $\dot{V}O_2$max (Table 1).

From the Departments of Medicine, Clinical Chemistry, and Anesthesiology and the Turku PET Centre, University of Turku, Turku; and Department of Medicine, Division of Endocrinology and Diabetology, University of Helsinki, Helsinki, Finland.

Submitted April 29, 1998; accepted July 7, 1998.

Supported by grants from the Finnish Academy of Science, Novo Nordisk Foundation, and Ahokainen Foundation.

Address reprint requests to P. Nuutila, MD, Department of Medicine, University of Turku, Kiinamyllynkatu 4-8, FIN-20520 Turku, Finland.

0026-0495/99/4802-0018$10.00/0

Table 1. Characteristics of the Study Groups

Characteristic	Basal Study		Insulin Clamp Study	
	Smokers (n = 14)	Nonsmokers (n = 23)	Smokers (n = 8)	Nonsmokers (n = 12)
Age (yr)	32 ± 9	32 ± 9	37 ± 10	38 ± 8
BMI (kg/m²)	23.7 ± 3.0	23.3 ± 2.9	24.9 ± 3.3	25.1 ± 2.8
$\dot{V}o_2max$ (mL/kg · min)	40.2 ± 7.0	41.2 ± 5.7	36.5 ± 6.5	39.5 ± 4.6
Systolic blood pressure (mm Hg)	122 ± 10	127 ± 8	124 ± 8	124 ± 6
Diastolic blood pressure (mm Hg)	79 ± 10	80 ± 9	77 ± 13	75 ± 9
Heart rate (1/min)	64 ± 13	62 ± 12	66 ± 11	65 ± 9
Fasting plasma glucose (mmol/L)	5.4 ± 0.5	5.4 ± 0.5	5.5 ± 0.5	5.4 ± 0.6
Fasting serum cholesterol (mmol/L)	5.0 ± 1.1	5.3 ± 0.8	5.3 ± 1.0	5.1 ± 0.6
Fasting serum HDL cholesterol (mmol/L)	1.2 ± 0.3	1.1 ± 0.2	1.0 ± 0.1	1.1 ± 0.2
Fasting serum triglycerides (mmol/L)	1.4 ± 0.7	1.0 ± 0.3	1.5 ± 0.7	1.0 ± 0.3
Blood hemoglobin (g/L)	148 ± 8	143 ± 5	152 ± 8	142 ± 3

All subjects were healthy as judged by the medical history, physical examination, and routine laboratory tests, and were not taking any medications. Written informed consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The experimental protocol was reviewed and approved by the Joint Ethical Committee of the Turku University Central Hospital and University of Turku.

Study Design

All subjects fasted overnight for 12 hours before the study and abstained from smoking during this period. The subjects remained supine during the study. Two catheters were inserted, one in an antecubital vein for infusion of glucose and insulin and injection of $[^{15}O]H_2O$ and [^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and one in the opposite radial artery for blood sampling. The study consisted of a 40-minute basal period and a 120-minute hyperinsulinemic period (Fig 1). The serum insulin concentration was increased using a primed-continuous (5 mU/kg · min) infusion of insulin for 120 minutes. Muscle blood flow in the femoral region was measured twice, once during the basal period and once after 60 minutes of hyperinsulinemia using $[^{15}O]H_2O$ and positron emission tomography (PET). Femoral muscle glucose uptake was measured immediately after the blood flow measurement using [^{18}F]FDG and PET. Whole-body glucose uptake was determined independently of the PET measurements using the euglycemic insulin clamp technique.[27]

Measurement of Muscle Blood Flow and Glucose Uptake With PET

For the flow studies, approximately 30 to 45 mCi $[^{15}O]H_2O$ was injected intravenously, and a dynamic scan was performed for 6 minutes. To obtain the input function, arterial blood was continuously withdrawn with a pump at a speed of 6 mL/min, and radioactivity was measured using a two-channel on-line detector system (Scanditronix, Uppsala, Sweden), which was cross-calibrated with an automatic gamma counter (Wizard 1480 3″; Wallac, Turku, Finland) and the PET scanner.

For the [^{18}F]FDG study, approximately 5 mCi [^{18}F]FDG was injected intravenously over 2 minutes and a 50-minute dynamic scan was started. Blood samples for measurement of plasma radioactivity were taken once during each time frame, and radioactivity was measured with an automatic gamma counter (Wizard 1480 3″).

Production of $[^{15}O]H_2O$ and [^{18}F]FDG. For production of [^{15}O] ($t_{½}$ = 123 seconds), a low-energy deuteron accelerator was used (Cyclone 3; Ion Beam Application, Louvain-la-Neuve, Belgium). $[^{15}O]H_2O$ was produced using a dialysis technique in a continuously working water module.[28] [^{18}F]FDG ($t_{½}$ = 109.8 minutes) was synthesized with an automatic apparatus as described by Hamacher et al.[29] Specific radioactivity at the end of the synthesis period was 2 Ci/μmol, and the radiochemical purity was greater than 98%.

Image acquisition and processing. An eight-ring ECAT 931/08 tomograph (Siemens/CTI, Knoxville, TN) was used. The scanner has an axial resolution of 6.7 mm and in-plane resolution of 6.5 mm.[30] The observed final in-plane resolution was 8 mm. The subject was positioned in the tomograph with the femoral regions in the gantry. Before the emission scan, a transmission scan for correction of photon attenuation was performed for 15 minutes with a removable ring source containing ^{68}Ge. All data were corrected for dead time, decay, and measured photon attenuation and reconstructed into a 128 × 128 matrix using a Hann filter with a cutoff frequency of 0.5.

Calculation of regional muscle blood flow. Internal and external dispersions were corrected for using an exponential dispersion function time constant, and the delay between the input curve and the tissue curve was solved by fitting. Blood flow was calculated pixel by pixel into flow images using the autoradiographic method[31,32] and a greater than 200-second tissue integration time as previously described.[32] The method for measuring blood flow with PET recently has been compared with venous occlusion plethysmography.[33]

Calculation of regional glucose uptake. The three-compartment model of [^{18}F]FDG kinetics[34] and a graphical analysis according to the method of Patlak and Blasberg[35] were used to quantify the fractional rate of tracer phosphorylation, K_i. The rate of glucose uptake, rGU, was obtained by multiplying K_i by the plasma glucose concentration, $[Glc]_p$, divided by a lumped constant term, LC: $rGU = K_i \times ([Glc]_p/LC)$. The LC accounts for differences in the transport and phosphorylation of [^{18}F]FDG and glucose, and was assumed to equal 1.0 for skeletal muscle as previously described.[36]

Regions of interest. Regions of interest (ROIs) were drawn in the anterior, anterolateral, and posterior muscle compartments of the

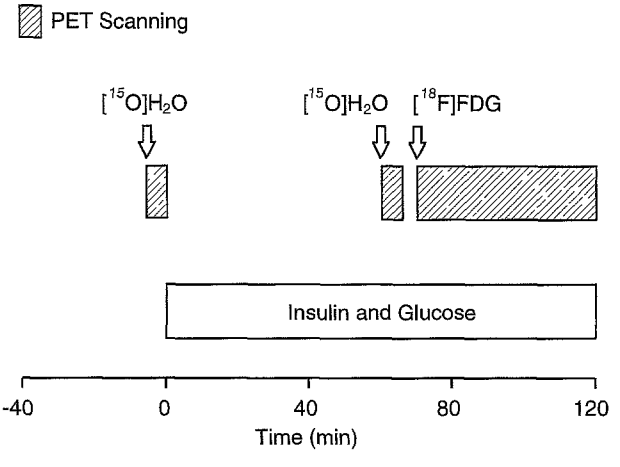

Fig 1. Study design. After a 40-minute basal period, insulin was infused intravenously at a rate of 5 mU/kg · min for 120 minutes and normoglycemia was maintained by glucose infusion at a variable rate. Muscle blood flow was measured in the basal state and after 60 minutes of insulin infusion with $[^{15}O]H_2O$ and PET. Insulin-stimulated glucose uptake was measured immediately after the second blood flow measurement with [^{18}F]FDG and PET.

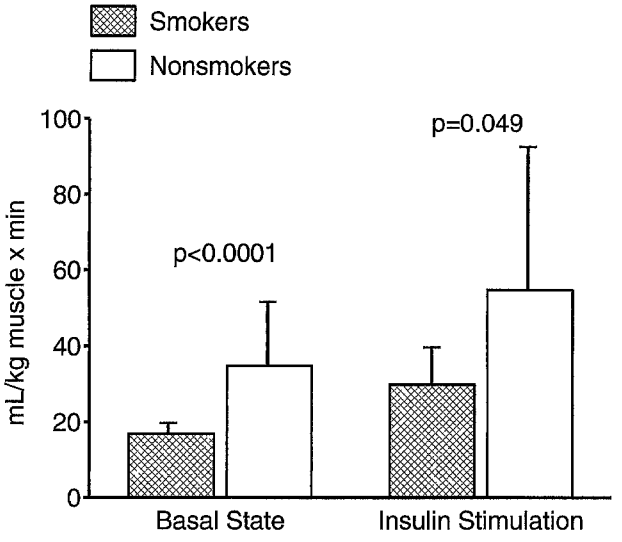

Fig 3. Femoral muscle blood flow in the basal state and during insulin stimulation in smokers and nonsmokers.

DISCUSSION

The novel finding of the present study is that basal femoral muscle blood flow was significantly lower in smokers compared with nonsmokers under fasting conditions. This finding was unexpected, since resting forearm blood flow, without exception, has been found to be comparable in smokers and nonsmokers as measured with venous occlusion plethysmography.[14,17,20,21] However, plethysmography only enables measurement of the total limb blood flow, not quantitation of regional blood flows within various tissues of the limb. If blood flow to nonmuscular tissue is substantially different from blood flow to muscle tissue, the total limb blood flow does not adequately reflect muscle blood flow. Blood flow per unit volume is higher in muscle versus, eg, subcutaneous fat and bone.[39,40] In addition, the relative forearm muscle content varies widely even in normal subjects[41] and has been found to be positively correlated with total forearm blood flow.[23] In general, smokers tend to be leaner than nonsmokers,[10,42] and smokers also have an altered body fat distribution favoring intraabdominal over peripheral subcutaneous fat.[42] Thus, if the smokers had relatively more muscle tissue in the forearm than nonsmokers, this may increase the apparent rate of total forearm blood flow and explain why a true difference in muscle blood flow might be missed. PET combined with $[^{15}O]H_2O$ offers the only technique presently available for the direct and reliable quantitation of muscle blood flow in humans. This method has been applied and validated for blood flow measurements in low-flow regions such as skeletal muscle by Ruotsalainen et al[32] in our laboratory.

Smoking has been associated with endothelial dysfunction of conduit and resistance arteries in several studies. For instance, abnormal responses to acetylcholine have been found in both the forearm[14] and coronary[15] circulation in heavy smokers. Flow-induced vasodilation, which is mediated via endothelial cell NO production in response to increased shear stress, is also impaired in healthy young white smokers.[16,43] In addition, the vasoconstrictive response to inhibition of endothelial cell NO synthase appears diminished in both peripheral[17,18] and coronary[19] vascular beds in smokers compared with nonsmokers, suggesting an impaired basal release and synthesis of NO by endothelial cells induced by smoking. However, the reduced basal production and release of NO and prostacyclin[17,18,44] in smokers might offer one explanation for the lower basal muscle blood flow in the smokers of the present study. Our subjects were young normotensive normolipidemic men with no clinical signs or symptoms of peripheral vascular disease. None of the subjects had any symptoms of myocardial or leg ischemia during the bicycle ergometer test. Therefore, it is unlikely that the reduced skeletal muscle blood flow in smokers was due to atherosclerotic manifestations in leg conduit arteries.

Insulin increases the total limb blood flow in a dose- and time-dependent fashion.[22,23] The insulin-induced vasodilatory effect is preferentially localized in skeletal muscle,[24] and the main mechanism responsible for this effect in humans seems to be the stimulation of NO synthesis in vascular endothelial cells.[25,26] We did not directly assess endothelial function in the present study. However, the similar percent increases in muscle blood flow in response to insulin in smokers and nonsmokers suggest that the stimulation of endothelial NO synthesis, at least by insulin, was not impaired in smokers.

The fasting serum insulin concentration, a marker of insulin

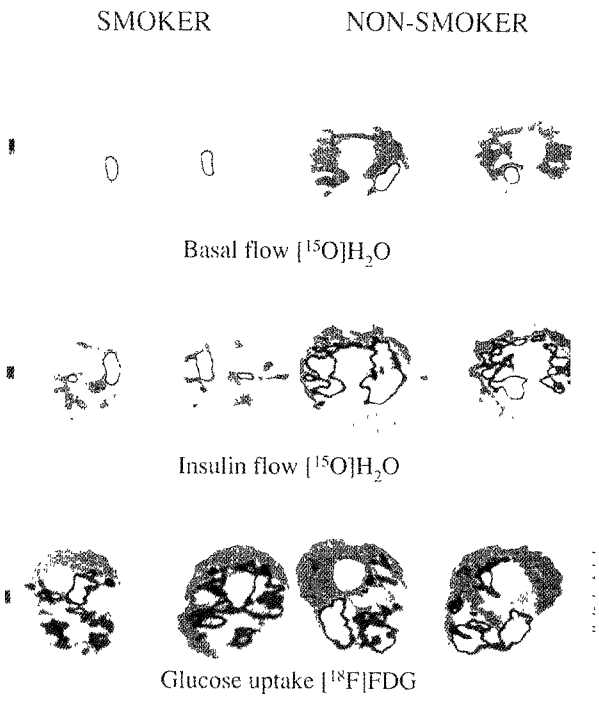

Fig 4. Parametric images showing basal (top) and insulin-stimulated (middle) muscle blood flow and insulin-stimulated muscle glucose uptake (bottom) in 1 smoker and 1 nonsmoker. The intensive red and yellow depict high blood flow or glucose uptake.

resistance,[45] has been found to be higher in smokers versus nonsmokers in some[10] but not all[8,9] studies. On the other hand, chronic smoking has not been observed to be associated with insulin resistance in studies in which insulin-stimulated whole-body glucose uptake has been measured using the "gold standard" euglycemic-hyperinsulinemic clamp technique either in normal subjects[9] or in patients with insulin-dependent diabetes mellitus.[46] In the present study, we found a slight difference (3 mU/L) in fasting serum insulin between smokers and nonsmokers. On the other hand, the insulin-stimulated rates of whole-body and skeletal muscle glucose uptake were not significantly different. This discrepancy may suggest that smokers can exhibit insulin resistance under low physiological insulin concentrations, and this resistance can be overcome by insulin concentrations that maximally stimulate glucose metabolism as applied in the present study. We chose a supraphysiological insulin concentration because it maximized the likelihood of detecting an insulin-induced increase in muscle blood flow and any putative defect in the vasodilatory response to insulin.[22,23]

The comparison of smokers and nonsmokers may be confounded by factors that determine insulin sensitivity in normal subjects,[12,13] such as the BMI and $\dot{V}O_2$max, independently of the smoking status. However, $\dot{V}O_2$max, perhaps the most important single determinant of insulin sensitivity,[13,47] has not been measured in previous studies. Therefore, the impaired insulin sensitivity observed in some studies in smokers[8,48] may be explained by differences in physical fitness. When we studied smokers and nonsmokers also matched for $\dot{V}O_2$max, we did not find any difference in insulin sensitivity.

It has been suggested but not proven that an insulin-induced increase in muscle blood flow may regulate insulin-stimulated glucose uptake in normal subjects, and that defects in the vasodilatory effect of insulin found in various insulin-resistant states[49] could contribute to the insulin resistance commonly observed in these patients. On the other hand, blood flow responses to insulin do not differentiate between insulin-resistant and -sensitive normal individuals.[50] An unchanged insulin stimulation of glucose uptake despite an approximately 85% lower rate of muscle blood flow in smokers versus nonsmokers in the present study strongly suggests that skeletal muscle can compensate for the long-term reduction in muscle perfusion by increasing glucose extraction. This concept is in agreement with previous studies showing that increased muscle blood flow induced by, eg, local bradykinin[33] or adenosine[51] infusion does not per se increase muscle glucose uptake, but is in contrast to studies in which acute reductions in blood flow resulted in decreased insulin-stimulated glucose uptake.[52,53] The present study therefore implies that acute and chronic reductions in muscle blood flow are not necessarily comparable with regard to the effect on insulin-stimulated muscle glucose uptake.

We conclude that muscle blood flow is lower in chronic smokers compared with nonsmokers both under fasting conditions and under hyperinsulinemic conditions that maximally stimulate glucose metabolism. The increase in muscle blood flow and glucose uptake by insulin appears normal, suggesting that the vasodilatory and metabolic effects of insulin are intact in smokers and the reduced muscle blood flow per se does not cause insulin resistance in these subjects.

ACKNOWLEDGMENT

We thank Hannu Sipilä, Mika Teräs, Tuula Tolvanen, Ulla Ruotsalainen, Vesa Oikonen, and Mika Klemetsö, as well as the nurses at the Turku PET Center, for skillful technical assistance and dedication throughout the study. We also thank Jukka Kapanen and the Paavo Nurmi Center for $\dot{V}O_2$max determinations.

REFERENCES

1. Kannel WB, D'Agostino RB, Belanger AJ: Fibrinogen, cigarette smoking, and risk of cardiovascular disease: Insights from the Framingham Study. Am Heart J 113:1006-1010, 1987

2. Cryer PE, Haymond MW, Santiago JV, et al: Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med 295:573-577, 1976

3. Berlin I, Cournot A, Renout P, et al: Peripheral haemodynamic effects of smoking in habitual smokers. A methodological study. Eur J Clin Pharmacol 38:57-60, 1990

4. Kool MJF, Hoeks APG, Struijker Boudier HAJ, et al: Short- and long-term effects of smoking on arterial wall properties in habitual smokers. J Am Coll Cardiol 22:1881-1886, 1993

5. Grassi G, Seravalle G, Calhoun DA, et al: Mechanisms responsible for sympathetic activation by cigarette smoking in humans. Circulation 90:248-253, 1994

6. Klein LW, Ambrose J, Pichard A, et al: Acute coronary hemodynamic response to cigarette smoking in patients with coronary artery disease. J Am Coll Cardiol 3:879-886, 1984

7. Trap-Jensen J: Effects of smoking on the heart and peripheral circulation Am Heart J 115:263-267, 1988

8. Facchini FS, Hollenbeck CB, Jeppesen J, et al: Insulin resistance and cigarette smoking. Lancet 339:1128-1130, 1992

9. Nilsson PM, Lind L, Pollare T, et al: Increased level of hemoglobin A_{1c}, but not impaired insulin sensitivity, found in hypertensive and normotensive smokers. Metabolism 44:557-561, 1995

10. Rönnemaa T, Rönnemaa EM, Puukka P, et al: Smoking is independently associated with high plasma insulin levels in nondiabetic men. Diabetes Care 19:1229-1232, 1996

11. Attvall S, Fowelin J, Lager I, et al: Smoking induces insulin resistance—Potential link with the insulin resistance syndrome. J Intern Med 233:327-332, 1993

12. Clausen JO, Borch-Johnsen K, Ibsen H, et al: Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young healthy caucasians. Analysis of the impact of gender, body fat, physical fitness, and life-style factors. J Clin Invest 98:1195-1209, 1996

13. Yki-Järvinen H Role of insulin resistance in the pathogenesis of NIDDM. Diabetologia 38:1378-1388, 1995

14. Heitzer T, Ylä-Herttuala S, Luoma J, et al: Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia. Role of oxidized LDL. Circulation 96:1346-1353, 1996

15. Niterberg A, Antony I, Foult JM: Acetylcholine-induced coronary vasoconstriction in young, heavy smokers with normal coronary arteriographic findings. Am J Med 95:71-77, 1993

16. Celermajer DS, Sorensen KE, Georgakopoulos D, et al: Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation 88:2149-2155, 1993

17. McVeigh GE, Lemay L, Morgan D, et al: Effects of long-term cigarette smoking on endothelium-dependent responses in humans. Am J Cardiol 78:668-672, 1997

18. Kiowski W, Linder L, Stoschitzky K, et al: Diminished vascular response to inhibition of endothelium-derived nitric oxide and enhanced vasoconstriction to exogenously administered endothelin-1 in clinically healthy smokers. Circulation 90:27-34, 1994

19. Kugiyama K, Yasue H, Ohgushi M, et al: Deficiency in nitric oxide bioactivity in epicardial coronary arteries of cigarette smokers. J Am Coll Cardiol 28:1161-1167, 1996

20. Rångemark C, Wennmalm Å: Endothelium-dependent and -independent vasodilation and reactive hyperemia in healthy smokers. J Cardiovasc Pharmacol 20:S198-S201, 1992 (suppl 12)

21. Jacobs M-C, Lenders JWM, Kapma JA, et al: Effect of chronic smoking on endothelium-dependent vascular relaxation in humans. Clin Sci 85:51-55, 1993

22. Laakso M, Edelman SV, Brechtel G, et al: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J Clin Invest 85:1844-1852, 1990

23. Utriainen T, Malmström R, Mäkimattila S, et al: Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. Diabetologia 38:555-564, 1995

24. Raitakari M, Nuutila P, Ruotsalainen U, et al: Evidence for dissociation of insulin stimulation of blood flow and glucose uptake in human skeletal muscle. Studies using [^{15}O]H_2O, [^{18}F]fluoro-2-deoxy-D-glucose, and positron emission tomography. Diabetes 45:1471-1477, 1996

25. Scherrer U, Randin D, Vollenweider P, et al: Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 94:2511-2515, 1994

26. Steinberg HO, Brechtel G, Johnson A, et al: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 94:1172-1179, 1994

27. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

28. Crouzel C, Clark J, Brihaye C, et al: Radiochemistry automation for PET, in Stöcklin G, Pike V (eds): Radiopharmaceuticals for Positron Emission Tomography. Dordrecht, The Netherlands, Kluwer, 1993, pp 45-90

29. Hamacher K, Coenen HH, Stöcklin G: Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 27:235-238, 1996

30. Spinks TJ, Jones T, Gilardi MC, et al: Physical performance of the latest generation of commercial positron scanners. IEEE Trans Nucl Sci 35:721-725, 1988

31. Howard BE, Ginsberg MD, Hassel WP, et al: On the uniqueness of cerebral blood flow measured by the in vivo autoradiographic strategy and positron emission tomography. J Cereb Blood Flow Metab 2:99-108, 1983

32. Ruotsalainen U, Raitakari M, Nuutila P, et al: Quantitative blood flow measurement of skeletal muscle using oxygen-15-water and PET. J Nucl Med 38:314-319, 1997

33. Nuutila P, Raitakari M, Laine H, et al: Role of blood flow in regulating insulin-stimulated glucose uptake in humans. Studies using bradykinin, [^{15}O]water and [^{18}F]fluoro-deoxy-glucose and positron emission tomography. J Clin Invest 97:1741-1747, 1996

34. Sokoloff L, Reivich M, Kennedy C, et al: The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897-916, 1977

35. Patlak CS, Blasberg RG: Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. J Cereb Blood Flow Metab 5:584-590, 1985

36. Nuutila P, Koivisto VA, Knuuti J, et al: Glucose–free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest 89:1767-1774, 1992

37. Stæhr P, Hother-Nielsen O, Levin K, et al: Is hepatic insulin resistance a major feature of NIDDM patients with peripheral insulin resistance? Diabetes 37:A136, 1994 (suppl 1, abstr)

38. Hother-Nielsen O, Henriksen JE, Holst JJ, et al: Effects of insulin on glucose turnover rates in vivo: Isotope dilution versus constant specific activity technique. Metabolism 45:82-91, 1996

39. Raitakari M, Nuutila P, Ruotsalainen U, et al: The relationship between limb and muscle blood flow in man. J Physiol 469:543-549, 1996

40. Utriainen T, Nuutila P, Takala T, et al: Intact insulin stimulation of skeletal muscle blood flow, its heterogeneity and redistribution but not of glucose uptake in non–insulin-dependent diabetes mellitus. J Clin Invest 100:777-785, 1997

41. Maughan RJ, Watson JS, Weir J: The relative proportions of fat, muscle and bone in the normal human forearm as determined by computed tomography. Clin Sci 66:683-689, 1984

42. Shimokata H, Muller DC, Andres R: Studies in the distribution of body fat. III. Effects of cigarette smoking. JAMA 261:1160-1173, 1989

43. Woo KS, Robinson JTC, Chook P, et al: Differences in the effect of cigarette smoking on endothelial function in Chinese white adults. Ann Intern Med 127:372-375, 1997

44. Zhu B-Q, Parmley WW: Hemodynamic and vascular effects of active and passive smoking. Am Heart J 130:1270-1275, 1995

45. Laakso M: How good a marker is insulin level for insulin resistance? Am J Epidemiol 137:959-965, 1993

46. Helve E, Yki-Järvinen H, Koivisto VA: Smoking and insulin sensitivity in type 1 diabetic patients. Metabolism 35:874-877, 1986

47. Rosenthal M, Haskell WL, Solomon R, et al: Demonstration of a relationship between level of physical training and insulin-stimulated glucose utilization in normal humans. Diabetes 32:408-411, 1983

48. Eliasson B, Attvall S, Taskinen M-R, et al: The insulin resistance syndrome in smokers is related to smoking habits. Arterioscler Thromb 14:1946-1950, 1994

49. Baron AD: Hemodynamic actions of insulin. Am J Physiol 267:E187-E202, 1994

50. Utriainen T, Mäkimattila S, Virkamäki A, et al: Dissociation between insulin sensitivity of glucose uptake and endothelial function in normal subjects. Diabetologia 39:1477-1482, 1996

51. Natali A, Bonadonna R, Santoro D, et al: Insulin resistance and vasodilation in essential hypertension. Studies with adenosine. J Clin Invest 94:1570-1576, 1994

52. Baron AD, Steinberg HO, Chaker H, et al: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. J Clin Invest 96:786-792, 1995

53. Ferrannini E, Taddei S, Santoro D, et al: Independent stimulation of glucose metabolism and Na^+-K^+ exchange by insulin in the human forearm. Am J Physiol 255:E953-E958, 1988