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Type II Diabetes Abrogates Sex Differences in Endothelial Function in Premenopausal Women

Helmut O. Steinberg, MD; Giancarlo Paradisi, MD; Jessica Cronin, RN; Kristin Crowde, RN; Annette Hempfling, RN; Ginger Hook, RN; Alain D. Baron, MD

Background—Obesity is a more potent cardiovascular risk factor (CVRF) in men than in women. Because traditional CVRFs cannot fully account for this sex difference, we tested the hypothesis that compared with men, women exhibit more robust endothelial function independent of obesity and that this sex difference is abrogated by diabetes.

Methods and Results—We studied leg blood flow (LBF) responses to graded intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride (Mch) and the endothelium-independent vasodilator sodium nitroprusside (SNP) in groups of lean, obese (OB), and type II diabetic (DM) premenopausal women and age- and body mass index-matched men. LBF response to intrafemoral administration of L-NMMA, an inhibitor of nitric oxide synthase, was also assessed in normal men and women. Maximum LBF increments in response to Mch were $347 \pm 57\%$ versus $231 \pm 22\%$ in lean women versus men ($P < 0.05$) and $203 \pm 25\%$ versus $111 \pm 17\%$ in OB women versus men ($P < 0.01$), respectively. In DM, maximum LBF increments in response to Mch were $104 \pm 24\%$ and $138 \pm 33\%$ in women and men, respectively, ($P = \text{NS}$). LBF decrements in response to L-NMMA were $34.9 \pm 4.1\%$ and $17.1 \pm 4.2\%$ in women and men, respectively ($P < 0.01$). The response to SNP was not different between sexes and groups.

Conclusions—Premenopausal nondiabetic women exhibit more robust endothelium-dependent vasodilation owing to higher rates of nitric oxide release than men. Given the protective vascular action of nitric oxide, this difference may partially explain the lower incidence of macrovascular disease in women. In premenopausal women, DM causes impairment of endothelial function beyond that observed with obesity alone and leads to endothelial dysfunction similar to that observed in DM men. These findings may help explain the similar rates of coronary artery disease and mortality in diabetic men and women. (*Circulation*. 2000;101:2040-2046.)

Key Words: endothelium ■ obesity ■ diabetes mellitus ■ sex

Obesity is considered an independent risk factor for macrovascular disease.^{1,2} Despite a higher prevalence of obesity in women,³ the incidence of macrovascular disease is significantly lower in premenopausal women than men even when matched for traditional cardiovascular risk factors (CVRFs). Obesity (particularly when centrally distributed) is characteristically accompanied by insulin resistance, which is associated with a cluster of CVRFs.⁴ Adding to the list of CVRFs, we recently reported impaired endothelium-dependent vasodilation (EDV) in healthy obese insulin-resistant subjects.⁵ On closer analysis of our data, we noted possible sex differences in EDV. Given the central role of the endothelium to modulate vascular tone, lipid peroxidation, smooth muscle proliferation and migration, and monocyte adhesion,⁶ sex differences in endothelial function could partially account for the sex differences in cardiovascular events previously noted. Therefore, we hypothesized that compared with men, women exhibit higher rates of basal

nitric oxide (NO)-dependent blood flow and more robust EDV independent of obesity. Furthermore, because the mortality from macrovascular disease is 3- to 5-fold higher in type II diabetic women^{7,8} than in nondiabetic women and reaches coronary event rates similar to those of diabetic men, we reasoned that if endothelial dysfunction is in fact an important CVRF, both diabetic men and women would exhibit similar degrees of endothelial dysfunction. In other words, we hypothesized that type II diabetes abrogates differences in endothelial function between sexes.

We studied leg blood flow (LBF) changes in response to graded intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride (Mch) and the endothelium-independent vasodilator sodium nitroprusside (SNP) in groups of lean, obese (OB), and OB type II diabetic (DM) men and women. Furthermore, to assess NO-dependent vascular tone, we measured LBF changes in response to the inhibitor of NO synthase *N*^G-monomethyl-L-arginine (L-NMMA).

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TABLE 1. Demographic and Metabolic Characteristics of the Study Groups Receiving Graded Intrafemoral Artery Infusions of Mch

	Men			Women		
	Lean	Obese	Type II Diabetes	Lean	Obese	Type II Diabetes
n	44	16	8	5	23	7
Age, y	33.5±1.0	34.1±1.8	39.1±2.1	34.4±3.4	33.9±1.8	36.0±2.9
Body mass index, kg/m ²	23.2±0.4	32.5±0.9*	32.3±2.4*	21.5±1.4	36.3±1.6*	35.8±2.6*
Percent body fat	17.8±0.8	32.0±1.1*	29.9±1.8*	25.1±2.6	46.5±1.4*	41.7±2.3*
Absolute body fat mass, kg	12.8±0.7	32.8±1.9*	31.1±5.3*	14.1±2.5	45.7±2.9*	39.2±3.7*
Waist/hip ratio	0.92±0.01	0.96±0.02	0.96±0.01	0.81±0.02	0.86±0.01	0.91±0.03†
Fasting glucose, mg/dL	93.7±0.9	94.6±3.2	186.4±28.2†	87.8±1.1	91.9±2.2	219.3±25.4†
Fasting insulin, μU/mL	6.4±0.5§	10.8±1.9	19.7±7.3†	10.3±1.5	13.1±1.6	17.4±5.0
Mean arterial pressure, mm Hg	87±1‡	97±2‡	106±6‡	87±2	95±2	93±2
Total cholesterol, mg/dL	163±5	189±8	193±11	158±15	163±6	192±19
Triglycerides, mg/dL	107±8	164±29*	163±24*	77±19	84±5	223±72†
HDL cholesterol, mg/dL	45±2	33±3*	35±3*	49±3	41±2	37±5
LDL cholesterol, mg/dL	101±4	111±9	131±8*	99±15	104±6	117±19
Fasting FFA, μmol/L	394±24‡	575±59‡	764±116‡	451±42	619±67	810±46*

Values are mean±SD.

Statistics within sex groups: * $P<0.05$ vs lean; † $P<0.05$ vs lean and obese; ‡ $P<0.05$ vs each other.

Statistics between men and women: § $P<0.05$ vs female group; || $P<0.01$ vs female group.

Methods

Study Population

Study subjects were healthy, with normal cuff blood pressure. DM subjects were withdrawn from oral antidiabetic drugs ≥ 4 weeks before studies. Long-acting and intermediate-acting insulin was stopped 1 week before the study, and regular insulin was withheld for a minimum of 24 hours before the study. Obesity was defined as body mass index (weight [kg]/(height [m²]) ≥ 26 in men and ≥ 28 in women. Studies were approved by the Indiana University Institutional Review Board, and all volunteers gave informed consent.

Protocol

All studies were done after an overnight fast. A 6F sheath (Cordis Corp) was placed into the right femoral vein to allow the insertion of a custom-designed 5F double-lumen thermodilution catheter (Baxter Scientific, Edwards Division) to measure LBF. The right femoral artery was cannulated with a 5.5F double-lumen catheter to allow simultaneous infusion of substances and invasive blood pressure monitoring via a vital signs monitor (Spacelabs).

All hemodynamic measurements were obtained with the subjects in the supine position in a quiet temperature-controlled room. Baseline LBF and mean arterial pressure measurements were obtained after ≥ 30 minutes of rest after the insertion of the catheters. Rates of LBF were obtained with the thermodilution technique and calculated by a cardiac output computer (model 9520A, American Edwards Laboratories). During baseline, 24 LBF measurements were obtained at ≈ 30 -second intervals. During drug infusion, LBF measurements were begun 2 minutes after the onset of each dose, and 10 measurements were taken for each dose. Invasively determined MAP was recorded with every other LBF determination.

Graded intrafemoral artery infusions of Mch or SNP (Roche Laboratories) were administered at sequential doses of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 $\mu\text{g}/\text{min}$ (0.1 to 0.6 mL/min) or 1.75, 3.5, and 7.0 $\mu\text{g}/\text{min}$ (0.25 to 1.0 mL/min) to assess stimulated endothelium-dependent or endothelium-independent vasodilation, respectively. Basal NO-dependent vasodilation was assessed by an intrafemoral artery infusion of L-NMMA (Clinalfa), an inhibitor of NO synthase,

at a dose of 16 mg/min for 15 minutes (2.0 mL/min). The responses to Mch, SNP, and L-NMMA were studied in separate groups.

Statistical Analysis

Comparison between groups was performed by factorial or repeated-measures ANOVA. When significant differences between groups were found by ANOVA, this was followed by post hoc testing with Fisher's protected least significant difference test. Because basal LBF differed significantly between groups, changes in blood flow are expressed as percent change (% Δ) to adjust for differences at baseline. Univariate regression analysis between the maximum changes in LBF (% Δ LBF) in response to Mch and other variables known to modulate this response was performed after transformation of $\Delta\%$ LBF to its square root. Statistical significance was accepted at a level of $P<0.05$. All results are shown as the mean±SEM.

Results

Mch Study: Endothelium-Dependent Vasodilation

Metabolic and baseline hemodynamic characteristics of the study group are shown in Table 1. As expected, the groups exhibited differences in these characteristics due to the presence of obesity and/or type II diabetes.

In the men, the increments in LBF (Figure 1A) were significantly reduced in the OB and DM subjects. Lean subjects exhibited a nearly 2-fold higher rise in LBF than both OB and DM subjects ($P<0.01$). Maximum LBF increments were 231±22%, 111±17%, and 138±33% in lean, OB, and DM, respectively ($P<0.05$ lean versus OB and DM). In the women, the increase in LBF (Figure 1B) was significantly reduced in OB and DM. However, although OB women had a significantly smaller response than the lean women, they still exhibited nearly twice the rise in LBF compared with DM ($P<0.05$). Maximum LBF increments were 347±57%, 203±25%, and 104±24% in lean, OB, and

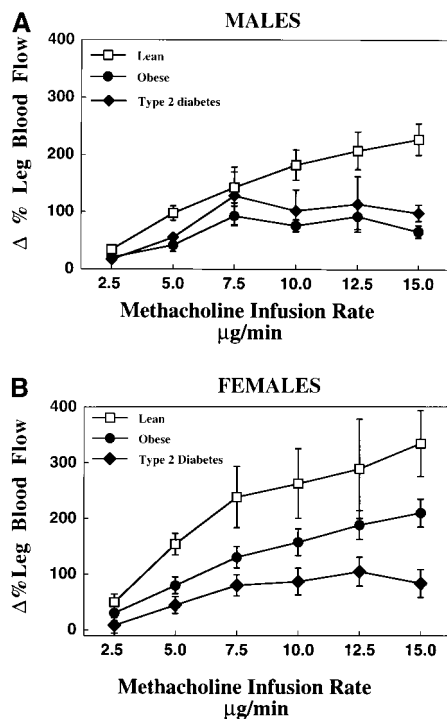


Figure 1. LBF increments above baseline (%Δ) in response to graded intrafemoral artery infusions of endothelium-dependent vasodilator Mch in lean, OB, and DM men (A) and in lean, OB, and DM women (B). In men, dose-response curves were different between lean and both OB and DM groups ($P<0.01$). In women, dose-response curves were different between all groups ($P<0.05$).

DM, respectively ($P<0.01$ between all groups). These data indicate that obesity and type II diabetes are associated with impaired endothelial function. Matching OB and DM women for waist-to-hip ratio (data not shown) did not attenuate the difference in LBF responses.

To further demonstrate that sex differences exist in the vascular response to Mch, we compared the LBF increments in response to Mch between sexes according to their groups (lean, OB, and DM). Lean women exhibited $\approx 40\%$ more pronounced increases in LBF (Figure 2A) than lean men, and the maximal LBF response to Mch was $347\pm 57\%$ versus $231\pm 22\%$ in female and male subjects, respectively ($P<0.05$). OB women exhibited nearly twice the LBF increments in response to Mch than the OB men (Figure 2B), and the maximal LBF response to Mch was $203\pm 25\%$ versus $111\pm 17\%$ in female and male subjects, respectively ($P<0.01$). Matching OB men and women for absolute body fat mass (data not shown) did not alter the results. In contrast to lean and OB women, DM women exhibited responses to Mch similar to those of DM men (Figure 2C), and maximal increments in LBF in response to Mch were $138\pm 33\%$ and $104\pm 24\%$ ($P=NS$) in men and women, respectively.

These data indicate that EDV is modulated by sex and is more robust in nondiabetic premenopausal women than in nondiabetic men and that this sex difference is abrogated by diabetes.

Univariate analysis between maximum response to Mch and other factors known to determine endothelial function,

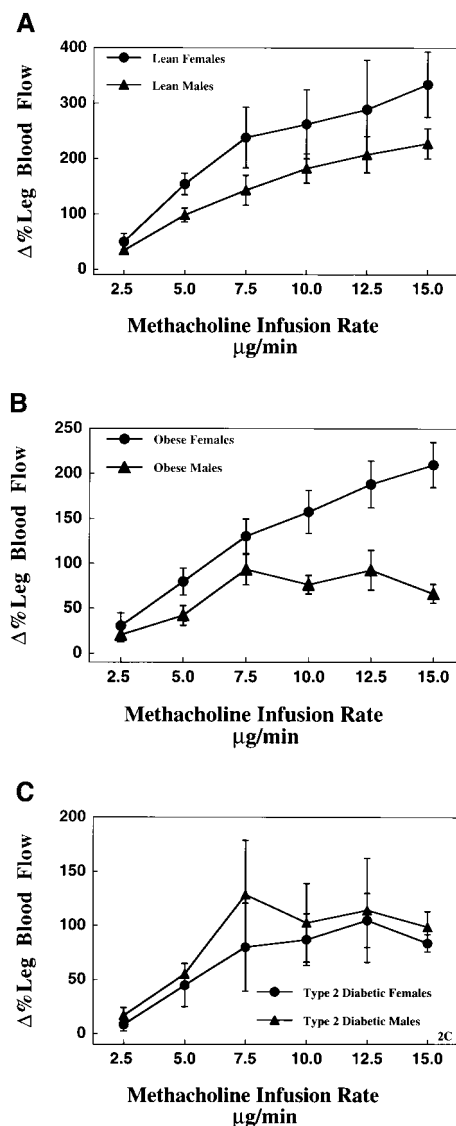


Figure 2. LBF increments above baseline (%Δ) in response to graded intrafemoral artery infusions of endothelium-dependent vasodilator Mch in groups of lean (A), OB (B), and DM (C) men and women. Dose-response curves between men and women were different for lean ($P=0.06$) and OB ($P<0.01$) but not DM groups.

such as indices of body fat content, blood pressure, age, and cholesterol, revealed that body mass index, percent body fat content, hip and waist circumference, absolute body fat mass, and free fatty acid (FFA) levels achieved statistical significance (Table 2). Exclusion of the diabetic subjects did not change the results of these analyses. Using multivariate or stepwise regression analysis did not alter this finding, indicating that body fat content, body fat distribution, and basal serum FFAs appear to be the most important determinants of endothelial dysfunction.

L-NMMA Study: Basal NO-Dependent Vasodilation

Metabolic and baseline hemodynamic characteristics of the study group are shown in Table 3.

In response to intrafemoral L-NMMA, LBF decreased to 0.169 ± 0.020 and 0.187 ± 0.025 L/min in the male and female

TABLE 2. Univariate Regression Analyses for the Relationship Between Maximum Response to Mch and Variables Known to Modulate This Response

Variable	Men		Women	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Body mass index	0.49	<0.0001	0.52	<0.001
Percent body fat content	0.50	<0.0001	0.32	0.058
Absolute fat mass	0.41	<0.001	0.43	0.01
Waist circumference	0.39	<0.01	0.57	<0.001
Hip circumference	0.39	<0.01	0.46	<0.01
FFA	0.3	<0.05	0.55	<0.01
Mean arterial pressure	0.09	NS	0.14	NS
Cholesterol	0.12	NS	0.22	NS
Fasting insulin	0.15	NS	0.26	NS
Age	0.18	NS	0.21	NS

All subjects including diabetics are included.

groups, respectively ($P<0.05$ versus basal, both groups). The fall in LBF in response to L-NMMA (Figure 3) was $17.1\pm 4.2\%$ and $34.9\pm 4.1\%$ in the male and female groups, respectively ($P<0.01$), indicating that women exhibit higher rates of basal NO production even in the face of higher body fat content (Table 3).

SNP Study:

Endothelium-Independent Vasodilation

Metabolic and baseline hemodynamic characteristics of the study group are shown in Table 4. As expected, the groups exhibited differences in these characteristics due to the presence of obesity and/or type II diabetes.

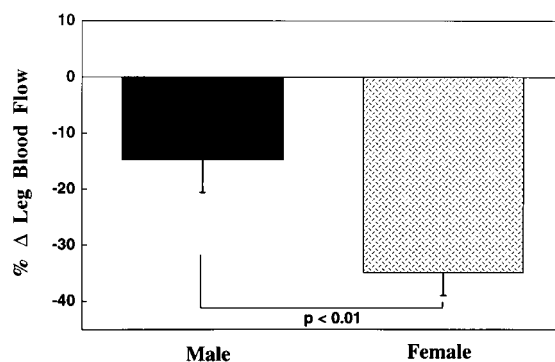
In both men and women, the LBF response to SNP was comparable between lean, OB, and DM subjects (Figure 4A and 4B). No differences were found in the LBF response to SNP between men and women. These results indicate that endothelium-independent vasodilation is not modulated by sex or impaired by obesity or type II diabetes.

TABLE 3. Demographic and Metabolic Characteristics of the Study Groups Receiving Intrafemoral Artery Infusions of L-NMMA

	Men	Women
n	8	8
Age, y	32.1 ± 2.1	33.0 ± 2.9
Body mass index, kg/m ²	28.2 ± 1.5	31.5 ± 2.4
Percent body fat	25.5 ± 1.3	$46.8\pm 2.4^*$
Fasting glucose, mg/dL	93.6 ± 2	89.0 ± 2.1
Fasting insulin, μ U/mL	6.1 ± 1.0	$11.9\pm 2.6^*$
Mean arterial pressure, mm Hg	88.2 ± 2.1	92.2 ± 4.1
Total cholesterol, mg/dL	198 ± 16	162 ± 6
Triglycerides, mg/dL	125 ± 16	88 ± 12
HDL cholesterol, mg/dL	43 ± 5	43 ± 6
LDL cholesterol, mg/dL	128 ± 18	97 ± 8

Values are mean \pm SD.

* $P<0.01$ men vs women.

**Figure 3.** LBF decrements (% Δ) in response to intrafemoral artery infusion of L-NMMA (16 mg/min), an inhibitor of NO synthase, in male and female subjects.

Discussion

Obesity is an independent risk factor for macrovascular disease across sexes.^{1,9,10} However, despite higher incidence of obesity in premenopausal women, rates of macrovascular disease are lower in premenopausal women than in men. Interestingly, this sex difference, which normally vanishes after menopause,¹¹ is rapidly lost in premenopausal DM patients, with cardiovascular disease reaching 2- to 5-fold higher rates than in nondiabetic women.⁸ In fact, women with type II diabetes, compared with age-matched nondiabetic women, exhibit 5-fold⁷ to 8-fold¹² higher rates of death related to coronary artery disease, with event rates nearly identical to those observed in type II diabetic men. Traditional CVRFs cannot completely account for these sex differences in cardiovascular mortality.¹³

The results of our study demonstrate that (1) NO-dependent basal vascular tone and EDV is enhanced in nondiabetic premenopausal women compared with men; (2) obesity/insulin resistance is associated with blunting of EDV in both sexes, and this effect is markedly more pronounced in men; (3) although type II diabetes in men does not further reduce EDV beyond that observed with obesity, diabetes in obese women causes impairment of EDV above and beyond that observed with obesity alone; and (4) diabetes was associated with similar endothelial dysfunction in women and men. Together, these results suggest that premenopausal women exhibit higher rates of NO production than men independent of obesity and that this sex difference is abrogated by type II diabetes. Moreover, obesity appears to have a more potent effect to diminish endothelial function in men than in women.

The increased effect of Mch in nondiabetic women could be due to (1) increased NO production/release, (2) increased NO action at the level of the vascular smooth muscle in the women, or (3) decreased NO action at the level of the vascular smooth muscle in the men. Differences in NO action between sexes are highly unlikely, because the effect of graded intrafemoral artery infusions of the exogenous NO donor SNP did not differ between sexes, indicating no differences in vascular smooth muscle cell responses to NO between sexes. Thus, our data indicate that the increased vasodilator response to Mch in nondiabetic premenopausal

TABLE 4. Demographic and Metabolic Characteristics of the Study Groups Receiving Graded Intrafemoral Artery Infusions of SNP

	Men			Women	
	Lean	Obese	Type II Diabetes	Obese	Type II Diabetes
n	18	5	3	5	5
Age, y	33.6±1.5	29.2±2.3	42.0±3.5‡	28.7±2.8	35.6±4.1
Body mass index, kg/m ²	23.4±0.7†	29.0±1.3	26.8±2.5	36.6±4.3	35.0±2.3
Percent body fat	18.3±1.3*	28.6±0.4§	29.9±1.9§	44.5±1.3	46.7±1.3
Fasting glucose, mg/dL	92.9±1.1	97.3±2.3	290.5±104.7‡	89.8±2.5	239.0±28.1†
Fasting insulin, μ U/mL	5.7±0.5†	10.7±1.7	6.2±0.8	12.0±2.4	16.6±6.5
MAP, mm Hg	88.4±1.6	90.2±3.3	112.9±17.3	97.0±4.1	98.4±3.8
Total cholesterol, mg/dL	176±7	184±24	177±21	154±4	193±11†
Triglycerides, mg/dL	120±13	188±36	133±34	91±14	147±17†
HDL cholesterol, mg/dL	39±3	27±5	38±6	40±4	36±2
LDL cholesterol, mg/dL	115±7	122±20	114±9	97±6	127±8
Fasting FFA, μ mol/L	453±40	593±126	946±245‡	580±77	943±134†

Values are mean±SD.

Statistics within sex groups: * P <0.01 vs both OB and DM; † P <0.05 vs OB; ‡ P <0.05 vs both lean and OB.

Statistics between men and women: § P <0.001 vs female group.

women is due, at least in large part, to increased production/release of NO.

Because vascular responses to muscarinic agonists like Mch or acetylcholine are not mediated exclusively by the release of NO,¹⁴ other vasodilating factors may mediate some of the vascular response. One of these, the endothelium-dependent hyperpolarizing factor, has been shown to contribute more to EDV in female than male rat mesenteric arter-

ies.¹⁵ Therefore, differences in endothelium-dependent hyperpolarizing factor release or action could theoretically also partially explain the sex differences in EDV.

We also examined basal NO-dependent vascular tone by measuring LBF response to intrafemoral artery administration of L-NMMA, an inhibitor of NO. Nondiabetic women, compared with nondiabetic men, exhibited \approx 2-fold greater LBF decrements in response to intrafemoral artery infusions of L-NMMA. These results indicate that women exhibit higher rates of basal NO production than men, as suggested by Forte et al,¹⁶ who measured whole-body NO production. This difference in NO-dependent vascular tone is all the more significant because both sex groups were well matched for factors known to impair EDV, such as age,¹⁷ blood pressure,¹⁸ and FFA¹⁹ and cholesterol²⁰ levels.

It is important to emphasize that, even when matched for body mass index, women tended to have significantly higher body fat content than men, which is consistent with other reports.^{21–23} Because obesity/insulin resistance has been shown by us⁵ and others²⁴ to be associated with impaired EDV, one would have predicted reduced LBF responses to both L-NMMA and Mch in women. Further studies will be necessary to determine whether fat distribution could account for these differences, because dual x-ray absorptiometry as used in this study does not quantify intra-abdominal adiposity. Nevertheless, our data support the notion that sex has a major modulating effect on endothelial function independent of obesity.

The precise mechanism(s) for the enhanced NO-dependent endothelial vasodilation in premenopausal nondiabetic women is unknown. Obviously, sex differences in sex hormones may be one explanation for the differences in NO production/release. In endothelial cell cultures, estrogen, the predominant female sex hormone, has been shown to stimulate NO synthesis.²⁵ Vascular strips from female rats were found to release more NO in response to acetylcholine than

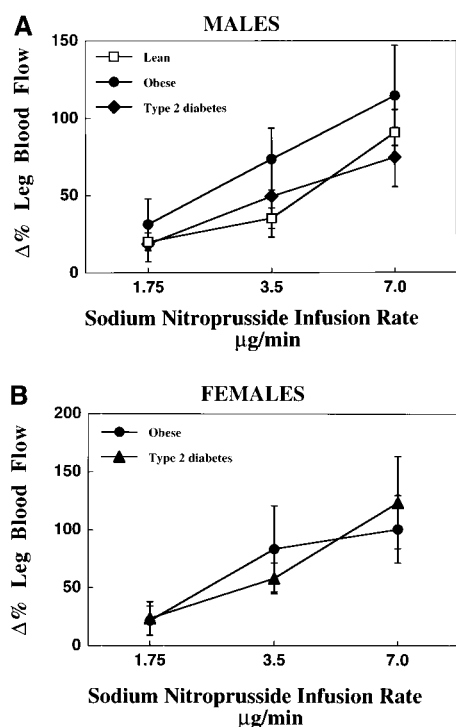


Figure 4. LBF increments above baseline ($\Delta\%$) in response to graded intrafemoral artery infusions of endothelium-independent vasodilator SNP in lean, OB, and DM men (A) and in OB and DM women (B).

vascular strips from male rats.^{26,27} In humans, estrogen replacement restored the blunted blood flow response to acetylcholine in women who underwent ovariectomy²⁸ and in postmenopausal women.²⁹ These data suggest that estrogen may directly stimulate NO production/release in women. Conversely, the predominant male sex hormone testosterone (or other androgens) may cause decreased NO production/release, as suggested by Herman and colleagues.³⁰ The independent contributions of estrogens and androgens to the control of endothelial function in normal and pathophysiological states remains to be fully elucidated.

In addition, differences in insulin sensitivity between men and women may also account for higher rates of NO production/release in women. Several reports have demonstrated that women display nearly 50% higher insulin sensitivity than men when matched for age and body mass index,^{22,23} suggesting that sex modulates the association between body fat content and insulin sensitivity. We⁵ and others²⁴ have shown that insulin sensitivity correlates positively with the magnitude of the blood flow responses to Mch and L-NMMA. Therefore, the greater endothelial dysfunction observed in men may be secondary to the more reduced insulin sensitivity in men versus women.

The mechanism(s) by which obesity impairs EDV is not well understood. Obesity is associated with elevated FFA,^{31,32} and we have previously shown¹⁹ that elevation of FFA in lean insulin-sensitive subjects caused blunting of EDV. Furthermore, elevation of FFA in vitro^{33,34} decreases NO production in endothelial cells. We found that FFA levels and the maximum response to Mch were significantly and inversely related. Taken together, our findings suggest that elevation of FFA levels in OB and OB DM subjects may be causally related to the observed endothelial dysfunction.

It is important to note that the presence of diabetes caused a further impairment of EDV in women but not in men. This suggests that obesity alone caused a maximal reduction in EDV in men and a submaximal effect in women. The slightly higher LDL cholesterol levels in the diabetic women may account for a small proportion (10% to 20%) of the difference in the LBF response to Mch. At the very least, our data further suggest that obesity is sufficient to cause endothelial dysfunction associated with decreased NO release in men, whereas superimposed hyperglycemia is necessary to produce similar degrees of endothelial impairment in woman.

Finally, it is important to consider the clinical implications of our data. Assuming that endothelial dysfunction is important in the development of macrovascular disease,³⁵ it follows that control of hyperglycemia would be expected to have only a modest effect to reduce macrovascular disease in men, because this intervention would have limited effects to improve insulin sensitivity (reverse insulin resistance) and thus would not benefit endothelial function. In contradistinction, therapeutic interventions to control hyperglycemia would be expected to have dramatic effects in premenopausal women, because this maneuver would be expected to greatly ameliorate endothelial function. Conversely, maneuvers directed at reducing both obesity/insulin resistance (weight loss, exercise, insulin-sensitizing drugs) and hyperglycemia would be expected to greatly reduce macrovascular disease in

both men and women. In this context, it is interesting that the results of the UK Prospective Diabetes Study³⁶ showed only marginal effects of glycemic control alone (without amelioration of insulin resistance) on macrovascular disease. Unfortunately, an analysis examining sex differences in these outcomes has yet to be presented.

In summary, obesity/insulin resistance is associated with impaired endothelial function (reduced NO release) in both sexes, but this effect is remarkably more pronounced in men. Given the vasoprotective properties of endothelium-derived NO, this relative preservation of endothelial function may explain, at least in part, the decreased incidence of hypertension and cardiovascular disease in nondiabetic premenopausal women. Importantly, the development of severe endothelial dysfunction in women with type II diabetes could explain the epidemiological finding that type II diabetic women and men display similar elevated cardiovascular risk.

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References

- Jousilahti P, Tuomilehto J, Vartiainen E, Pekkanen J, Puska P. Body weight, cardiovascular risk factors, and coronary mortality: 15-year follow-up of middle-aged men and women in eastern Finland. *Circulation*. 1996;93:1372-1379.
- Seidell JC, Verschuren WM, van Leer EM, Kromhout D. Overweight, underweight, and mortality: a prospective study of 48,287 men and women. *Arch Intern Med*. 1996;156:958-963.
- From the Centers for Disease Control and Prevention. Update: prevalence of overweight among children, adolescents, and adults—United States, 1988-1994. *JAMA*. 1997;277:1111.
- Reaven GM. Syndrome X: 6 years later. *J Intern Med*. 1994;236:13-22.
- Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. *J Clin Invest*. 1996; 97:2601-2610.
- Lloyd-Jones DM, Bloch KD. The vascular biology of nitric oxide and its role in atherogenesis. *Annu Rev Med*. 1996;47:365-375.
- Pan WH, Cedres LB, Liu K, Dyer A, Schoenberger JA, Shekelle RB, Stamler R, Smith D, Collette P, Stamler J. Relationship of clinical diabetes and asymptomatic hyperglycemia to risk of coronary heart disease mortality in men and women. *Am J Epidemiol*. 1986;123: 504-516.
- Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. *JAMA*. 1979;241:2035-2038.
- Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26 year follow up of participants in the Framingham Heart Study. *Circulation*. 1983;67: 968-977.
- Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH, Speizer F. Body weight and mortality among women. *N Engl J Med*. 1995;333:677-685.
- Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med*. 1976;85:447-452.
- Jarrett RJ, McCartney P, Keen H. The Bedford survey: ten year mortality rates in newly diagnosed diabetics, borderline diabetics and normoglycaemic controls and risk indices for coronary heart disease in borderline diabetics. *Diabetologia*. 1982;22:79-84.
- Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev*. 1987;3:463-524.
- Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A. Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest*. 1997;100:2793-2799.

15. McCulloch AI, Randall MD. Sex differences in the relative contributions of nitric oxide and EDHF to agonist-stimulated endothelium-dependent relaxations in the rat isolated mesenteric arterial bed. *Br J Pharmacol*. 1998;123:1700–1706.
16. Forte P, Kneale BJ, Milne E, Chowienczyk PJ, Johnston A, Benjamin N, Ritter JM. Evidence for a difference in nitric oxide biosynthesis between healthy women and men. *Hypertension*. 1998;32:730–734.
17. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension*. 1996;27:849–853.
18. Panza JA, Quyyumi A, Brush JE, Epstein SE. Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990;323:22–27.
19. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest*. 1997;100:1230–1239.
20. Casino PR, Kilcoyne CM, Quyyumi AA, Hoeg JM, Panza JA. The role of nitric oxide in endothelium-dependent vasodilation of hypercholesterolemic patients. *Circulation*. 1993;88:2541–2547.
21. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr*. 1991;65:105–114.
22. Nuutila P, Knuuti MJ, Maki M, Laine H, Ruotsalainen U, Teras M, Haaparanta M, Solin O, Yki-Jarvinen H. Gender and insulin sensitivity in the heart and in skeletal muscles: studies using positron emission tomography. *Diabetes*. 1995;44:31–36.
23. Yki-Jarvinen H. Sex and insulin sensitivity. *Metabolism*. 1984;33:1011–1015.
24. Petrie JR, Ueda S, Webb DJ, Elliott HL, Connell JMC. Endothelial nitric oxide production and insulin sensitivity: a physiological link with implications for pathogenesis of cardiovascular disease. *Circulation*. 1996;93:1331–1333.
25. Lantin-Hermoso RL, Rosenfeld CR, Yuhanna IS, German Z, Chen Z, Shaul PW. Estrogen acutely stimulates nitric oxide synthase activity in fetal pulmonary artery endothelium. *Am J Physiol*. 1997;273:L119–L126.
26. Kauser K, Rubanyi GM. Gender difference in bioassayable endothelium-derived nitric oxide from isolated rat aortae. *Am J Physiol*. 1994;267:H2311–H2317.
27. Huang A, Sun D, Koller A, Kaley G. Gender difference in flow-induced dilation and regulation of shear stress: role of estrogen and nitric oxide. *Am J Physiol*. 1998;275:R1571–R1577.
28. Pinto S, Virdis A, Ghiadoni L, Bernini G, Lombardo M, Petraglia F, Genazzani AR, Taddei S, Salvetti A. Endogenous estrogen and acetylcholine-induced vasodilation in normotensive women. *Hypertension*. 1997;29:268–273.
29. Tagawa H, Shimokawa H, Tagawa T, Kuroiwa-Matsumoto M, Hirooka Y, Takeshita A. Short-term estrogen augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilation in postmenopausal women. *J Cardiovasc Pharmacol*. 1997;30:481–488.
30. Herman SM, Robinson JTC, McCredie RJ, Adams MR, Boyer MJ. Androgen deprivation is associated with enhanced endothelium dependent-vasodilation in adult men. *Arterioscler Thromb Vasc Biol*. 1997;17:2004–2009.
31. Herranz L, Zapata A, Grande C, Megia A, Pallardo LF. Body fat distribution, insulin mediated suppression of non-esterified fatty acids and plasma triglycerides in obese subjects. *Horm Metab Res*. 1998;30:141–145.
32. Boden G. Free fatty acids (FFA), a link between obesity and insulin resistance. *Front Biosci*. 1998;3:D169–D175.
33. Gupta MP, Steinberg H, Baron A, Hart CM. Fatty acids impair nitric oxide production in cultured endothelial cells. *J Invest Med*. 1998;46:288A. Abstract.
34. Davda RK, Stepniakowski KT, Lu G, Ullian ME, Goodfriend TL, Egan BM. Oleic acid inhibits endothelial nitric oxide synthase by a protein kinase C-independent mechanism. *Hypertension*. 1995;26:764–770.
35. Cannon RO III. Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin Chem*. 1998;44:1809–1819.
36. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998;352:837–853.