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## Myocardial insulin resistance associated with chronic hypertriglyceridemia and increased FFA levels in Type 2 diabetic patients

Lucilla D. Monti,<sup>1</sup> Claudio Landoni,<sup>2</sup> Emanuela Setola,<sup>3</sup> Elena Galluccio,<sup>1</sup> Pietro Lucotti,<sup>3</sup> Emilia P. Sandoli,<sup>1</sup> Anna Origgi,<sup>3</sup> Giovanni Lucignani,<sup>4</sup> PierMarco Piatti,<sup>3</sup> and Ferruccio Fazio<sup>2</sup>

<sup>1</sup>Core Lab, Diabetology, Endocrinology, and Metabolic Disease Unit, Medicine Division, <sup>2</sup>Division of Nuclear Medicine,

<sup>3</sup>Cardiovascular and Metabolic Rehabilitation Unit, Rehabilitation and Functional Reeducation Division, Istituto di Recupero e Cura a Carattere Scientifico Hospital San Raffaele 20132 Milan; and <sup>4</sup>Imaging and Radiotherapy Unit, University of Milan, I-20090 Milan, Italy

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**Monti, Lucilla D., Claudio Landoni, Emanuela Setola, Elena Galluccio, Pietro Lucotti, Emilia P. Sandoli, Anna Origgi, Giovanni Lucignani, PierMarco Piatti, and Ferruccio Fazio.** Myocardial insulin resistance associated with chronic hypertriglyceridemia and increased FFA levels in Type 2 diabetic patients. *Am J Physiol Heart Circ Physiol* 287: H1225–H1231, 2004. First published May 6, 2004; 10.1152/ajpheart.00629.2003.— We evaluated the influence of chronic hypertriglyceridemia and endothelial dysfunction on myocardial glucose uptake (MGU) in Type 2 diabetic patients without coronary heart disease. Patients were divided into two groups according to fasting triglyceride (TG) levels:  $5.4 \pm 1.1$  and  $1.5 \pm 0.3$  mmol/l for high- and normal-TG groups, respectively. Five subjects were assigned to the high-TG group and 11 to the normal-TG group. Age, gender, body mass index, systolic and diastolic blood pressure, glucose, insulin, HbA1c, cholesterol, and HDL cholesterol were similar in the two groups, whereas free fatty acid (FFA) levels were higher in the high-TG group basally and at the end of the clamp. Furthermore, five healthy subjects were subjected to the same protocol and used as the control group. MGU was assessed by using <sup>18</sup>F-labeled 2-fluoro-2-deoxy-D-glucose under hyperglycemic-hyperinsulinemic conditions. Basal endothelin-1 and nitric oxide levels were significantly higher in the high-TG group than in the normal-TG and control groups, and cGMP and maximal postischemic vasodilation were significantly decreased in the high-TG group compared with the normal-TG and control groups. However, significant alterations were found in the same parameters in the normal-TG group compared with the control group. By the end of the hyperglycemic clamp, in the high-TG group, MGU was ~40 and 65% of that in the normal-TG and control groups. MGU negatively correlated with TG, FFA, and endothelin-1, whereas a positive correlation was found with cGMP and maximal postischemic vasodilation. In conclusion, increased TG and FFA levels are risks, in addition to Type 2 diabetes mellitus, for myocardial insulin resistance, endothelial dysfunction, and alteration of nitric oxide/cGMP levels.

triglycerides; myocardial glucose uptake; positron emission tomography study; endothelium; free fatty acid

FOR MANY YEARS, hypertriglyceridemia was considered a marker of cardiovascular disease only in certain subjects, such as women (5, 12) and patients with Type 2 diabetes (10). The association between triglycerides (TGs), independently of HDL cholesterol levels, and definite cardiovascular disease, such as myocardial infarction or angina, was found in some studies (5) and denied in others, in which the simultaneous

association of high TG and low HDL cholesterol was necessary to predict cardiovascular risk (8). This apparent discrepancy is probably related to the fact that it is quite difficult to match for duration and degree of cardiovascular disease in different populations, and, when cardiovascular disease is established, some factors might have lost their primary pathogenic significance. Hypertriglyceridemia occurs frequently in patients with coronary heart disease (CHD) and in survivors of myocardial infarction (5), and, during the postprandial period, hypertriglyceridemia (13) is an independent risk factor for myocardial infarction in newly diagnosed Type 2 diabetic patients.

One possible explanation could be that high TG levels, a common feature in Type 2 diabetic patients, could decrease myocardial glucose uptake (MGU), with a detrimental effect on myocardial glucose oxidation and, in turn, on myocardial performance. It is well known that an increase in myocardial  $\beta$ -oxidation induced by high free fatty acid (FFA) and TG concentrations decreases heart glucose oxidation with a reduction in myocardial recovery after acute myocardial ischemia (18, 21). In the light of these data, recently our laboratory evaluated the effect of increased TGs in isolated hearts perfused under hyperglycemic-hyperinsulinemic conditions. Hearts were then exposed to 60 min of low-flow ischemia followed by 30 min of reperfusion. We demonstrated that increased TG levels impaired diastolic and systolic recovery, and this was paralleled by a simultaneous dose-response increase in endothelin (ET)-1 and nitric oxide (nitrite + nitrate, NO<sub>x</sub>) release. Interestingly, a  $\beta$ -oxidation inhibitor, trimetazidine, restored the contractile recovery, markedly increasing NO<sub>x</sub> and normalizing ET-1 levels (21).

Starting from these data, it might be postulated that high TG levels influence myocardial glucose metabolism and myocardial function by an inhibition of myocardial glucose metabolism and/or induction of endothelial dysfunction.

Therefore, the purpose of this cross-sectional study was to establish an association between high TG levels and endothelial function in MGU in Type 2 diabetic patients in the absence of CHD.

For this reason, MGU was assessed by using <sup>18</sup>F-labeled 2-fluoro-2-deoxy-D-glucose (<sup>18</sup>F]FDG) under hyperglycemic-hyperinsulinemic conditions. We selected hyperglycemic and hyperinsulinemic conditions to mimic the metabolic situation in Type 2 diabetic patients during the postprandial period and because basal MGU is extremely low and difficult to measure,

Address for reprint requests and other correspondence: L. D. Monti, Laboratory L20, Core Lab, Diabetology, Endocrinology, and Metabolic Disease Unit, Medicine Div., IRCCS Hospital San Raffaele, Via Olgettina 60, 20132 Milan, Italy (E-mail: lucilla.monti@hsr.it).

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especially in the high-TG group. In addition, circulating endothelial markers and postischemic vasodilation were measured in all patients to evaluate the presence of endothelial dysfunction and to evaluate the contribution of increased TGs to these alterations.

## MATERIALS AND METHODS

**Subjects.** Sixteen patients (3 women and 13 men) with Type 2 diabetes [age =  $48 \pm 2$  yr, body mass index (BMI) =  $26.4 \pm 1.3$  kg/m<sup>2</sup>, systolic blood pressure =  $132 \pm 4$  mmHg, diastolic blood pressure =  $84 \pm 3$  mmHg; HbA1c =  $6.5 \pm 0.4\%$ ] were recruited from the outpatient diabetes clinic. Type 2 diabetic patients had a negative history of cardiovascular disease, normal heart rate, and normal resting ECG and M-mode and two-dimensional echocardiogram. All patients were treated with diet alone. The duration of the diabetic disease averaged  $5 \pm 2$  yr.

On the basis of TG levels, patients were divided into two groups: a normal-TG group, consisting of 5 patients with TG levels of  $1.5 \pm 0.3$  (range 0.69–2.13) mmol/l, and a high-TG group, consisting of 11 patients with TG levels of  $5.4 \pm 1.1$  (range 3.14–12.46) mmol/l ( $P < 0.02$  vs. normal-TG group).

Five healthy subjects (age =  $46 \pm 1$  yr, BMI =  $25.8 \pm 1.3$  kg/m<sup>2</sup>, systolic blood pressure =  $104 \pm 2$  mmHg, diastolic blood pressure =  $74 \pm 2$  mmHg, TG =  $1.4 \pm 0.3$  mmol/l) served as controls.

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 Ethical Committee of Istituto Scientifico H San Raffaele.

**Study design.** All subjects were studied after a 10- to 12-h overnight fast. On the morning of each test, a 20-gauge catheter (Abbotcath, Venisystem, Abbot Ireland, Sligo, Ireland) was inserted into an antecubital vein for infusions. To sample arterialized blood, a second cannula was inserted anterogradely in a dorsal vein of the contralateral hand, and the hand was kept in a Plexiglas box heated at 55°C.

During the hyperglycemic clamp, an arterial plasma glucose concentration of  $\sim 10$  mmol/l was induced and maintained by a primed continuous infusion of 20% glucose [coefficient of variation (CV) < 5%]. Approximately 120 min of relatively constant hyperglycemia were allowed before the administration of [<sup>18</sup>F]FDG to initiate the procedure for determining the regional myocardial glucose utilization. The PET procedure lasted 97 min.

From the start of the hyperglycemic clamp to the end of the PET study, blood samples were taken every 30 min for measurement of serum insulin and plasma FFA. After the administration of [<sup>18</sup>F]FDG, serial arterialized blood samples were collected for 1 min and then at 1.5, 3.0, 5.0, 7.5, 10, 15, 25, 35, 45, 60, 75, 90, and 97 min to assess <sup>18</sup>F plasma concentration.

**PET studies.** PET studies were performed with a tomograph (ECAT 931/04-12, Siemens/CPS, Knoxville, TN). After 120 min of hyperglycemia, the subject was positioned supine on the bed of the tomograph. Then each subject received an intravenous pulse of 250 MBq of [<sup>18</sup>F]FDG over 2 min, and dynamic scanning was carried out. A 3-min transmission scan was performed at the end of the dynamic scan to compensate for movements and misalignment between transmission and emission scans. Transverse slices (128 × 128 matrix, zoom 3, 1.565-mm pixel, 6.75 mm thick) were reconstructed for each frame of the dynamic scan using the Hann filter with a cutoff frequency of 0.5 cycle/pixel. Data were corrected for <sup>18</sup>F decay and for attenuation. Under these conditions, the spatial resolution was 8 mm full width at half-maximum.

Images, reconstructed by processing transmission and dynamic scans, were used to outline the myocardial wall. Dynamic images were analyzed on a Sun workstation (SPARC). Three to four left ventricle transaxial slices were selected on the last frame of the dynamic emission scan; 9–12 irregular regions of interest (ROIs) were drawn around myocardial segments and then transferred to the

remaining images to obtain the time course of total radioactivity in each ROI. Data collected from the same myocardial segments in different planes were averaged and corrected for partial volume using a recovery coefficient calculated experimentally from a phantom study. A standard myocardial wall thickness of 10 mm was assumed for all the myocardial segments selected. In the case of low tracer uptake, ROIs were drawn on reprocessed transmission images, as previously described, and then transferred to the dynamic emission images.

**Data analysis.** MGU was calculated by graphic analysis of plasma and tissue time-activity curves (25). Lumped constant values of 1.0 were used, according to recently published data (3, 14, 22).

Whole body glucose uptake was measured from the glucose infusion rate (M value) in hyperglycemia during the PET scanning period (120–217 min).

Because insulin levels achieved during the hyperglycemic period were slightly different among the three groups ( $532 \pm 48$ ,  $448 \pm 61$ , and  $542 \pm 36$  pmol/l in high-TG, normal-TG, and control groups, respectively), we chose the measurement of the M value divided by the steady-state insulin levels during the same clamp period (M/I) as an index of peripheral insulin sensitivity.

**Measurement of postischemic vasodilation.** On the day before the hyperglycemic clamp, each patient underwent a postischemic vasodilation test. After an overnight fast and 30 min of rest, forearm blood flow (FBF) was measured from the left forearm by venous occlusion strain gauge plethysmography (27). Venous occlusion pressure averaged 60 mmHg in the cuff placed around the upper arm. Circulation to the hand was prevented by inflation of a pediatric cuff around the wrist to suprasystolic pressures (200 mmHg). After the blood flow measurements were completed (average of 4 flow curves), the upper arm was inflated to 50 mmHg systolic blood pressure for 5 min to induce ischemia. Maximal postischemic vasodilation was measured according to Capaldo et al. (4) and Raitakari et al. (30).

**Assays.** Plasma glucose was determined by a glucose oxidase method (Glucose Analyzer II, Beckman, Fullerton, CA). Serum insulin levels were assayed with a microparticle enzyme immunoassay (MEIA, IMX, Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

Serum TGs and FFAs were measured by automated enzymatic spectrofluorometric methods adapted to COBAS FARA II (Hoffman-La Roche, Basel, Switzerland).

ET-1 samples were extracted on a Sep-Pac C<sub>18</sub> minicolumn (Am-prep, Amersham, Buckinghamshire, UK). The eluate was evaporated in a Speed VAC SC110 (Savant Instruments, Farmingdale, NY). Samples were then reconstituted with 250 μl of RIA buffer and assayed by an RIA kit (NEN Life Science Products, Boston, MA) with an intra-assay CV of 3.0% and interassay CV of 11.9%.

cGMP was assayed with a RIA kit (Amersham).

NOx levels were evaluated through the measurement of metabolic end products, i.e., nitrite and nitrate via enzymatic catalysis coupled with the Griess reaction, as previously reported (35).

**Statistical analysis.** Values are means ± SD. Comparisons among groups were performed by one-way analysis of variance followed by Scheffé's *F* test when indicated. Linear regression was calculated using Pearson's correlation analysis. TG levels were logarithmically transformed to correct for skewness, and these variables were back-transformed to their natural units in Tables 1 and 3. Two-tailed  $P < 0.05$  was considered statistically significant.

## RESULTS

**Comparison of hormonal and metabolic variables and endothelial parameters between high-TG, normal-TG, and control groups in the basal state.** The three groups were matched for age and BMI, while systolic and diastolic blood pressures were similarly increased in the two groups of patients. Conversely, the high-TG group showed significantly higher fasting

Table 1. Clinical, hormonal, and metabolic characteristics of patients and controls

	High-TG Group	Normal-TG Group	Control Group
Gender, M/F	9/2	4/1	5/0
Age, yr	48±2	47±2	46±1
Body wt, kg	73.1±4.9	70.0±7.2	72.8±6.1
BMI, kg/m <sup>2</sup>	27.2±1.0	25.4±1.5	25.8±1.3
Blood pressure, mm Hg			
Systolic	135±3†	129±5†	104±2
Diastolic	84±2†	84±4†	74±2
Plasma glucose, mmol/l	6.8±0.5†	6.3±0.5†	5.5±0.2
Serum insulin, pmol/l	66.0±7.8†	49.8±11.4	44.4±11.4
Glycated Hb, %	7.3±0.4	6.2±0.3	
Serum TG, mmol/l	5.4±1.1*†	1.5±0.3	1.4±0.3
Plasma FFA, mmol/l	0.81±0.07*†	0.54±0.07	0.55±0.08
Cholesterol, mmol/l			
Total	4.50±0.30	4.70±0.37	4.40±0.25
HDL	1.38±0.34	1.44±0.33	1.56±0.15

Values are means ± SD. TG, triglyceride; BMI, body mass index; FFA, free fatty acid; M, male; F, female. \* $P < 0.05$  vs. normal-TG group. † $P < 0.05$  vs. control group.

TG ( $5.4 \pm 1.1$  vs.  $1.5 \pm 0.3$  and  $1.4 \pm 0.3$  mmol/l,  $P < 0.02$ ) and FFA levels ( $0.81 \pm 0.07$  vs.  $0.54 \pm 0.07$  and  $0.55 \pm 0.08$  mmol/l,  $P < 0.05$ ), while glucose, insulin, cholesterol, and HDL cholesterol were similar in the three groups (Table 1).

Endothelial parameters and postischemic hyperemic vasodilation values are reported in Table 2. Basal ET-1 ( $10.9 \pm 1.9$  and  $6.0 \pm 0.8$  vs.  $3.3 \pm 0.8$  pg/ml,  $P < 0.05$ ) and NOx levels ( $41.1 \pm 3.6$  and  $27.5 \pm 4.3$  vs.  $16.3 \pm 1.7$   $\mu\text{mol/l}$ ,  $P < 0.05$ ) were significantly higher than in the control group, with the highest values in the high-TG group. cGMP levels were significantly lower ( $2.28 \pm 0.24$  vs.  $3.86 \pm 0.24$   $\mu\text{mol/ml}$ ,  $P < 0.05$ ) in the high-TG group than in the normal-TG and control groups ( $4.9 \pm 0.48$   $\mu\text{mol/ml}$ ,  $P < 0.05$ ).

Basal FBF was similar in the three groups, while maximal postischemic FBF vasodilation showed the lowest levels in the high-TG group ( $16.7 \pm 1.2$  vs.  $21.3 \pm 1.2$  and  $28.4 \pm 3.9$  ml·100 ml<sup>-1</sup>·min<sup>-1</sup>,  $P < 0.05$ ).

*Myocardial and whole body insulin utilization during the hyperglycemic clamp combined with PET study.* By the end of the hyperglycemic clamp, the three groups of patients achieved similar steady-state glucose and insulin levels (Table 3). At the end of the clamp, FFA levels were threefold higher in the high-TG group than in the normal-TG and control groups ( $0.24 \pm 0.05$  vs.  $0.08 \pm 0.02$  and  $0.09 \pm 0.01$  mmol/l,  $P < 0.05$ ) and cGMP levels slightly increased in the two groups compared with basal levels but were 43% lower in the high-TG than in the normal-TG group ( $2.71 \pm 0.28$  vs.  $4.77 \pm 0.27$   $\mu\text{mol/ml}$ ,  $P < 0.0005$ ). As expected, the control group showed the highest cGMP increase ( $6.42 \pm 0.33$   $\mu\text{mol/ml}$ ,  $P < 0.05$  vs. high- and normal-TG groups).

Interestingly, MGU levels were ~40% lower in the high-TG than in the normal-TG group ( $452 \pm 80$  vs.  $1,042 \pm 133$   $\mu\text{mol}\cdot\text{kg muscle}^{-1}\cdot\text{min}^{-1}$ ,  $P < 0.05$ ) and 65% lower than in the control group ( $1,303 \pm 33$   $\mu\text{mol}\cdot\text{kg muscle}^{-1}\cdot\text{min}^{-1}$ ,  $P < 0.05$ ; Table 3). However, MGU was also significantly decreased in the normal-TG group compared with the control group (Table 3).

The M value, the index of peripheral glucose disposal derived at the end of the clamp, was significantly decreased

in the high-TG group compared with the normal-TG and control groups. Likewise, M/I, the index of whole body insulin sensitivity normalized by peripheral insulin levels at the end of the clamp, was decreased by 50% in the high-TG group ( $6.5 \pm 4.1 \times 10^2$  vs.  $13.1 \pm 4.9 \times 10^2$   $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{pmol}^{-1}\cdot\text{l}^{-1}$ ,  $P < 0.05$ ) and was threefold lower than in the control group ( $19.0 \pm 5.7 \times 10^2$   $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{pmol}^{-1}\cdot\text{l}^{-1}$ ,  $P < 0.05$  vs. the other two groups).

*Relations among metabolic and endothelial variables.* TG levels negatively correlated with basal cGMP levels ( $r = -0.66$ ,  $P < 0.002$ ; Fig. 1A) but positively correlated with NOx ( $r = 0.82$ ,  $P < 0.001$ ; not shown) and ET-1 ( $r = -0.63$ ,  $P < 0.002$ ; not shown).

At the end of the clamp, cGMP levels positively correlated with maximal postischemic FBF ( $r = 0.73$ ,  $P < 0.0001$ ; Fig. 1B). In addition, significant correlations were also found with ET-1 ( $r = -0.66$ ,  $P < 0.01$ ; not shown) and NOx levels ( $r = -0.85$ ,  $P < 0.0001$ ; not shown). FFA levels significantly correlated with maximal postischemic FBF ( $r = -0.63$ ,  $P < 0.002$ ; not shown).

*Relations among myocardial and whole body glucose utilization indexes and endothelial and metabolic variables.* A significant correlation was found between MGU and TG levels ( $r = -0.74$ ,  $P < 0.0001$ ; Fig. 2A) and between MGU and M/I ( $r = 0.67$ ,  $P < 0.01$ ; Fig. 2B).

Relations between MGU and cGMP at the end of the clamp ( $r = 0.86$ ,  $P < 0.001$ ), between MGU and maximal postischemic FBF ( $r = 0.72$ ,  $P < 0.003$ ), and between MGU and ET-1 ( $r = -0.64$ ,  $P < 0.002$ ) are shown in Fig. 3.

A significant correlation was found between FFA and cGMP levels ( $r = -0.62$ ,  $P < 0.002$ ; Fig. 4A) and between FFA levels and MGU ( $r = -0.60$ ,  $P < 0.005$ ; Fig. 4B).

M/I (an index of whole body insulin sensitivity) correlated with TG levels ( $r = -0.68$ ,  $P < 0.001$ ; not shown), FFA levels ( $r = -0.70$ ,  $P < 0.001$ ; not shown), and basal cGMP levels ( $r = 0.60$ ,  $P < 0.01$ ; not shown).

## DISCUSSION

The present study demonstrated a significant decrement in myocardial and peripheral glucose utilization in patients with Type 2 diabetes, hypertriglyceridemia, and increased FFA levels compared with Type 2 diabetic patients matched for all the other clinical and metabolic variables but with normal TG levels. These results were obtained despite the fact that the metabolic evaluation was performed in patients subjected to

Table 2. Basal endothelial variables and basal and postischemic hyperemic vasodilation in patients and controls

	High-TG Group	Normal-TG Group	Control Group
ET-1, pmol/ml	10.9±1.9*†	6.0±0.8†	3.3±0.8
NOx, $\mu\text{mol/l}$	41.1±3.6*†	27.5±4.3†	16.3±1.7
cGMP, $\mu\text{mol/ml}$	2.28±0.24*†	3.86±0.24†	4.90±0.48
FBF, ml·100 ml <sup>-1</sup> ·min <sup>-1</sup>			
Basal	2.4±0.2	2.3±0.3	2.9±0.6
Maximum postischemic	16.7±1.7*†	21.3±3.7†	28.4±3.9

Values are means ± SD. ET-1, endothelin-1; NOx, nitric oxide (nitrite + nitrate); FBF, forearm blood flow. \* $P < 0.05$  vs. normal-TG group. † $P < 0.05$  vs. control group.

Table 3. Steady-state metabolic variables, cGMP, and myocardial and whole body insulin sensitivity values at the end of hyperglycemic clamp combined with PET study

	High-TG Group	Normal-TG Group	Control Group
SSPG, mmol/l	10.8±0.3	10.4±0.2	10.5±0.1
SSSI, pmol/l	532±48	448±61	542±36
SSPFFA, mmol/l	0.24±0.05*†	0.08±0.02	0.09±0.01
SSSTG, mmol/l	4.5±0.9*†	1.0±0.3	0.9±0.2
cGMP, μmol/ml	2.71±0.28*†	4.77±0.27†	6.42±0.33
MGU, μmol·kg muscle <sup>-1</sup> ·min <sup>-1</sup>	452±80*†	1,042±133†	1,303±33
M value, μmol·kg <sup>-1</sup> ·min <sup>-1</sup>	32.6±5.9*†	55.5±7.4†	103.6±8.7
M/I, μmol·kg <sup>-1</sup> ·min <sup>-1</sup> ·pmol <sup>-1</sup> ·l <sup>-1</sup> × 10 <sup>2</sup>	6.8±2.5*†	13.4±4.9†	19.0±5.7

Values are means ± SD. SSPG, SSSI, SSPFFA, and SSSTG, steady-state plasma glucose, serum insulin, plasma FFA, and serum TG, respectively; MGU, myocardial glucose uptake; M value, glucose infusion rate; M/I, M value divided by steady-state insulin level during the same clamp. \*P < 0.05 vs. normal-TG group. †P < 0.05 vs. control group.

hyperglycemia-hyperinsulinemia in an attempt to mimic post-prandial metabolic conditions. It is well known that in normal subjects during this period the increase in insulin and glucose levels determines a shift toward the utilization of the glycolytic pathway (2, 9). In addition, myocardial glucose utilization was positively correlated with cGMP (the second messenger of NOx) and maximal postischemia-induced hyperemic vasodilation while negatively correlated with TG and ET-1 levels.

Effects of Type 2 diabetes mellitus on myocardial insulin resistance and endothelial dysfunction. In the present study, Type 2 diabetic patients with normal TG levels showed a significantly decreased MGU compared with the control group. This is not new and has been previously reported in Type 2

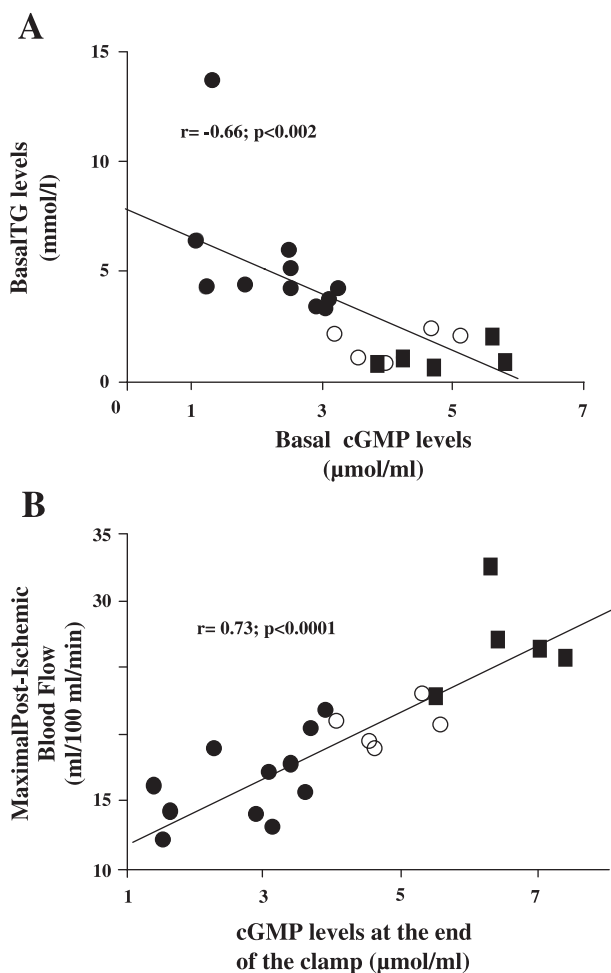


Fig. 1. Basal triglyceride (TG) vs. basal cGMP levels (A) and cGMP at the end of the clamp vs. cGMP after ischemic vasodilation (B) in Type 2 diabetic patients with high (●) and normal (○) TG levels and control subjects (■).

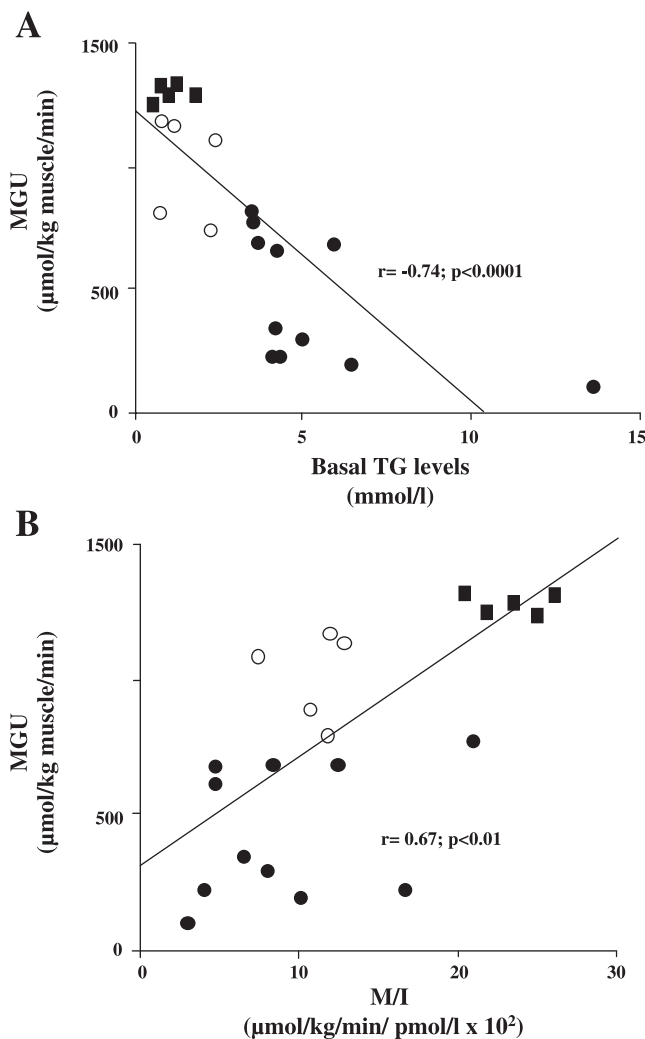


Fig. 2. Myocardial glucose uptake (MGU) vs. TG (A) and MGU vs. index of peripheral insulin sensitivity [whole body glucose uptake normalized to steady-state insulin level (M/I), B] in Type 2 diabetic patients with high (●) and normal (○) TG levels and control subjects (■).

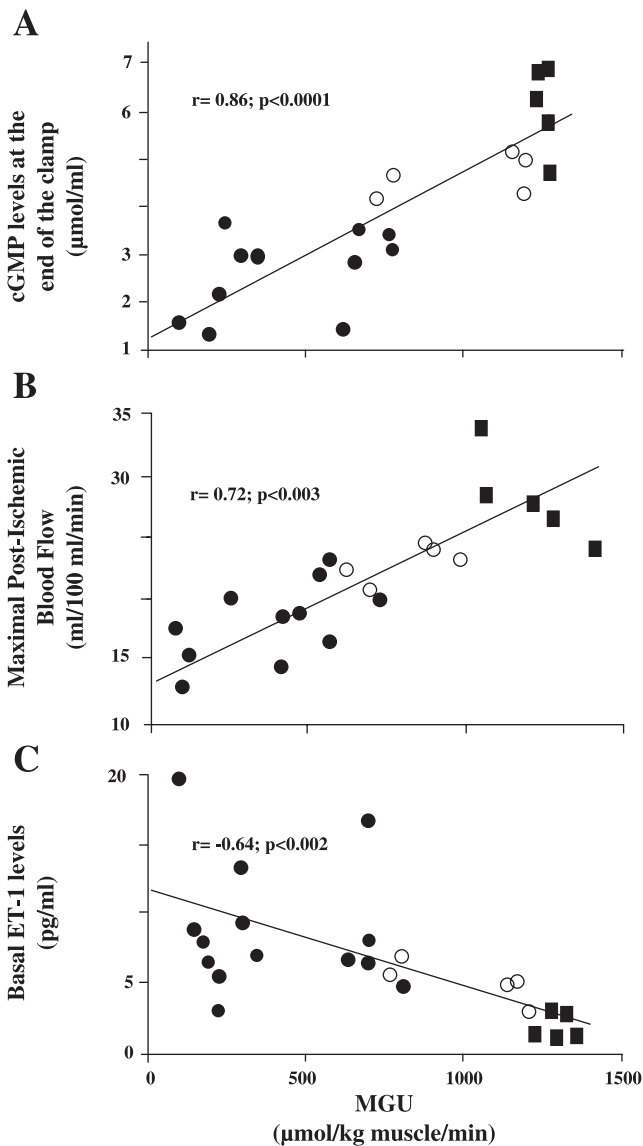


Fig. 3. MGU vs. cGMP at the end of the clamp (A), MGU vs. postischemic vasodilation (B), and MGU vs. basal endothelin-1 (ET-1, C) in Type 2 diabetic patients with high (●) and normal (○) TG levels and control subjects (■).

diabetic patients without cardiovascular disease by Iozzo et al. (14), who demonstrated in euglycemic-hyperinsulinemic subjects a severe myocardial insulin resistance, because insulin-mediated glucose uptake was reduced by 41%. Interestingly, a similar reduction of MGU was achieved also by our group in patients subjected to hyperglycemia-hyperinsulinemia, whereas in the presence of increased TG levels a reduction of ~65% was also achieved compared with the control group. In addition, recently Ceriello and Motz (6) reviewed research evidence indicating that postprandial hyperglycemia is directly implicated in the development of cardiovascular disease. Ceriello et al. (7) suggested that postprandial glucose may be directly involved in cardiovascular complications through a toxic effect on the vascular endothelium, inducing endothelial dysfunction, and that this atherogenic effect appears to be independent of other cardiovascular risk factors such as hyperlipidemia. According to these findings, in patients with Type 2

diabetes mellitus and normal TGs, we showed a decreased maximal postischemic FBF and an impairment of endothelial indexes, such as increased ET-1 levels and decreased cGMP levels, suggesting the presence of endothelial dysfunction compared with controls. Previous studies have reported the importance of Type 2 diabetes and hyperlipidemia as risk factors for CHD. In a 7-yr follow-up study in diabetic patients, the simultaneous presence of high fasting glucose with low HDL cholesterol and high total TGs significantly increased the risk for coronary events up to threefold, suggesting that dyslipidemia and poor glycemic control predict coronary mortality and morbidity in patients with Type 2 diabetes (15).

*Additive effects of hypertriglyceridemia and increased FFA levels on myocardial glucose utilization in Type 2 diabetic patients.* Fatty acids are important substrates for the heart (1), and FFA use is increased in the diabetic heart as a result of the high plasma FFA concentrations (see Ref. 37 for review).

Inside the myocytes, the increased concentrations of fatty acids will impair myocardial function through increased  $\beta$ -oxidation and decreased glucose oxidation, as initially demon-

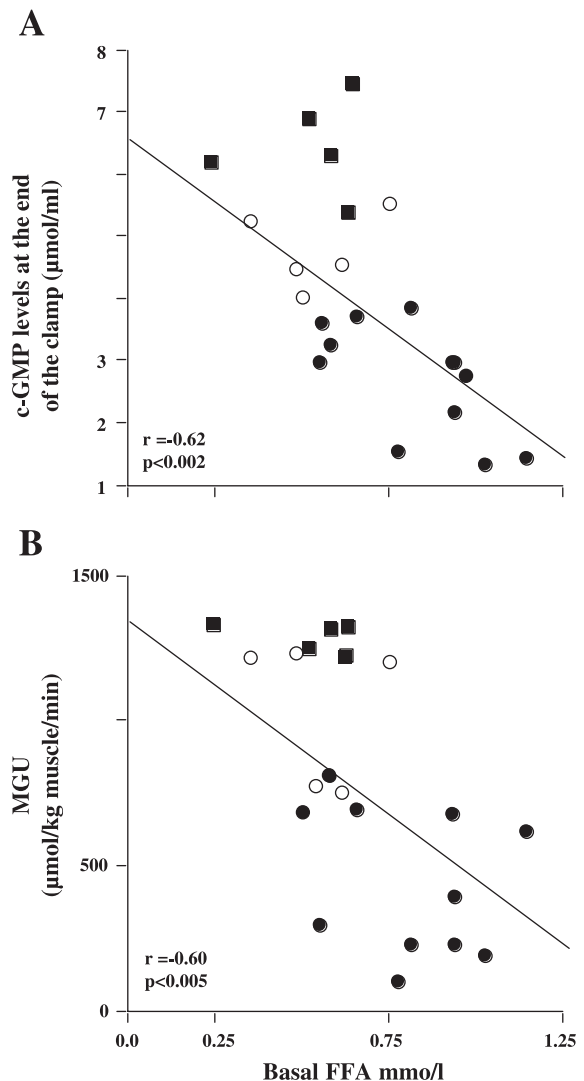


Fig. 4. Free fatty acid (FFA) levels vs. cGMP (A) and FFA vs. MGU (B) in Type 2 diabetic patients with high (●) and normal (○) TG levels and control subjects (■).

strated by Randle et al. (31) and confirmed by Lopaschuk et al. (17). Recently, it has been shown that increased myocardial  $\beta$ -oxidation induced by high FFA and TG concentrations decreased heart glucose oxidation and myocardial recovery after acute myocardial ischemia (18, 21). In particular, as reported elsewhere (21), under hyperglycemia-hyperinsulinemia, an acute elevation of TG levels in the medium shifted the hearts from carbohydrate to lipid metabolism with a concomitant impairment in postischemic recovery of systolic and diastolic functions. Our data seem to reinforce these findings, because patients with Type 2 diabetes and high TG levels had increased FFA levels in the fasting state and at the end of the hyperglycemic clamp, suggesting that the decreased MGU by high TGs relates to a Randle effect of FFA competition against glucose in the heart.

In addition, reduced MGU might be related to an excessive availability of TGs and fatty acids, which may exceed the rate of their use by the heart, resulting in lipid accumulation within the cardiomyocyte. This will result in the phenomenon of lipotoxicity, inducing reactive oxygen species accumulation, apoptosis, and contractile dysfunction of the heart (38).

*Effects of hypertriglyceridemia and increased FFA levels on endothelial function and its correlation with MGU.* In the present study, in the presence of insulin resistance and hypertriglyceridemia in Type 2 diabetic patients, fasting NOx levels are increased while cGMP levels are decreased, with a severe impairment in the ability of NOx to activate its messenger, as previously reported. Previous studies have shown that cGMP levels were significantly lower in first-degree relatives of patients with Type 2 diabetes independent of the degree of glucose tolerance (29) and in Type 2 diabetic patients than in normal subjects (28). Furthermore, maximal postischemic vasodilation was severely impaired, suggesting a marked degree of endothelial dysfunction.

These are confirmatory results of previous reports showing that insulin resistance syndrome is associated with endothelial dysfunction characterized by decreased NOx bioactivity and subnormal endothelium-dependent vasodilation. An excessive exposure to FFAs was postulated to be one of the mechanisms involved, because FFA overexposure, in vivo and in vitro, decreases the capacity of endothelial cells to generate bioactive NOx. In addition, the endothelial dysfunction tends to correlate with poor suppressability of FFA flux postprandially, and it has been proposed that TGs are synthesized and stored in endothelial cells during the postprandial period, when FFA and insulin are jointly elevated (19). In the present study, which mimics the postprandial conditions of increased glucose, insulin, TG, and FFA levels, a significant correlation was found between TG and FFA levels and maximal postischemic FBF. These findings seem to reinforce the detrimental role of excessive FFAs and TGs on the endothelium during the postprandial period.

Hyperinsulinemia is the other well-known player, acting in the induction of endothelial dysfunction in the presence of insulin resistance syndrome, typical of Type 2 diabetes and dyslipidemia. It is known that insulin acts at the level of the endothelial cells to modulate NO production/release, and this insulin effect is blunted in obese diabetic patients. The failure to increase endothelium-dependent vasodilation in response to hyperinsulinemia suggests that in these patients the endothe-

lium may be resistant to insulin's modulating effect on NO production/release (33).

More recently, Ceriello et al. (7) gave an interesting demonstration of the mechanism through which postprandial hyperglycemia and hypertriglyceridemia produce endothelial dysfunction, suggesting that it is related to the induction of an oxidative stress, supported by an increase of nitrotyrosine and by an increased inactivation of NO by O<sub>2</sub>. This also might be an indirect explanation of our findings of a staircase increase of NOx levels in patients with Type 2 diabetes without or with hypertriglyceridemia but decreased cGMP levels, suggesting a defective NO activity at the endothelial level.

Another important result was the finding of increased ET-1 levels in hypertriglyceridemic Type 2 diabetic patients. It is well known that increased ET-1 levels are present in insulin-resistant subjects, in Type 2 diabetic hyperlipidemic patients, and in atherosclerosis and cardiovascular disease (16, 20, 32). Moreover, in patients with coronary artery disease, a marked increase of ET-1 release was observed during exercise, in the presence of increased FFA levels (11).

In the present study, a negative correlation was found between ET-1 and MGU, supporting previous data demonstrating that in the presence of high TGs a dose-dependent increase in ET-1 release is achieved, accompanied with an impairment of postischemic recovery (21). In addition, ET-1 actively participates in the infarction size evolution of the ischemic heart (36), and plasma ET levels are an independent predictor of 1-yr mortality after acute myocardial infarction (24).

In an attempt to reconcile present and previously published data, it is important to underline the different study designs, because we have evaluated MGU during insulin stimulation and not in the fasting state. Moreover, as in previous studies (26), we cannot rule out the single or synergistic contribution of increased ET-1 levels or decreased NOx activity in reducing cGMP levels and decreasing MGU in our study population, because in vivo these mechanisms are similar, and it is quite difficult to rule out different roles. This important issue is beyond the scope of the present study and deserves further investigation.

*Clinical implications of the study.* The clinical implications of the present study support the role of high TGs and FFAs in the development of long-term adverse effects on myocardial function reported by Nuutila et al. (23).

The goal of the present study was to show alterations in myocardial glucose metabolism in two homogeneous groups of Type 2 diabetic patients in which all the metabolic variables except TG levels were strictly matched. Moreover, the patients were subjected to stable and comparable hyperglycemia and hyperinsulinemia to avoid the confounding effects on myocardial glucose metabolism related to different glucose and insulin levels.

The quite complex study design and the need to strictly match patients limited the number of patients in the study, but the metabolic and endothelial results were quite strong, suggesting the possibility that our results could be extended to Type 2 diabetic patients with high TG levels.

*Conclusions.* In conclusion, the present study demonstrated that increased TG and FFA levels are to be considered additional risks, in addition to Type 2 diabetes mellitus, for myocardial insulin resistance associated with endothelial dysfunction and alteration of NO/cGMP levels. This is important for

understanding diabetic cardiomyopathy, because hyperglycemia and high TG and FFA levels are common metabolic conditions in fasting and, furthermore, in the postprandial state in Type 2 diabetic patients.

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