# Simultaneous GLP-1 and Insulin Administration Acutely Enhances Their Vasodilatory, Antiinflammatory, and Antioxidant Action in Type 2 Diabetes

*Antonio Ceriello,[1] Anna Novials,[1] Silvia Canivell,[1] Lucia La Sala,[1] Gemma Pujadas,[1] Katherine Esposito,[2] Roberto Testa,[3] Loredana Bucciarelli,[4] Maurizio Rondinelli,[4] and Stefano Genovese[4]*

*Diabetes Care 2014;37:1938–1943 | DOI: 10.2337/dc13-2618*

**OBJECTIVE**

**To test the hypothesis that the simultaneous administration of GLP-1 and insulin may increase their vasodilatory, antiinflammatory, and antioxidant action in type 2 diabetes.**

**RESEARCH DESIGN AND METHODS**

**In two groups of persons with type 2 diabetes, two sets of experiments were performed. The first group had two normoglycemic-normoinsulinemic clamps with or without GLP-1 and two normoglycemic-hyperinsulinemic clamps with or without GLP-1. The second group had two hyperglycemic-normoinsulinemic clamps and two hyperglycemic-hyperinsulinemic clamps with or without GLP-1.**

**RESULTS**

**During the normoglycemic-hyperinsulinemic clamp, flow-mediated dilatation (FMD) increased, while soluble intercellular adhesion molecule (sICAM-1), plasma 8-iso-prostaglandin F2α (8-iso-PGF2α), nitrotyrosine, and interleukin (IL)-6 decreased compared with normoglycemic-normoinsulinemic clamp. Similar results were obtained with the infusion of GLP-1 during the normoglycemic-normoinsulinemic clamp. The combination of hyperinsulinemia and GLP-1 in normoglycemia was accompanied by a further FMD increase and sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 decrease. During the hyperglycemic-normoinsulinemic clamp, FMD significantly decreased, while sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 significantly increased. When hyperglycemia was accompanied by hyperinsulinemia or by the simultaneous infusion of GLP-1, these phenomena were attenuated. The simultaneous presence of hyperinsulinemia and GLP-1 had an increased beneficial effect.**

**CONCLUSIONS**

**Our results show that the combination of insulin and GLP-1 is more effective than insulin or GLP-1 alone in improving endothelial dysfunction, inflammation, and oxidative stress in type 2 diabetes.**

*[1]Insititut d'Investigacions Biomèdiques August Pi i Sunyer and CIBERDEM, Hospital Clínic Barcelona, Barcelona, Spain*
*[2]Division of Metabolic Diseases, Center of Excellence for Cardiovascular Diseases, 2nd University of Naples, Naples, Italy*
*[3]Metabolic and Nutrition Research Center on Diabetes, Italian National Research Center on Aging, Istituto Nazionale Riposo e Cura Anziani–Istituto di Ricovero e Cura a Carattere Scientifico, Ancona, Italy*
*[4]Department of Cardiovascular and Metabolic Diseases, Istituto di Ricovero e Cura a Carattere Scientifico Gruppo Multimedica, Sesto San Giovanni, Italy*

*Corresponding author: Antonio Ceriello, aceriell@clinic.ub.es.*

*Received 9 November 2013 and accepted 15 January 2014.*

Recent evidence has demonstrated that insulin has a vasodilatory and anti-inflammatory action in humans (1). One of the possible mechanisms involved in these actions seems to be related to the antioxidant property of insulin (2,3).

Apart from the well-documented incretin effect of glucagon-like peptide 1 (GLP-1), its role in the cardiovascular system also arouses interest. GLP-1 has a direct action on the endothelium (4), and it has been demonstrated that it improves endothelial function (5,6) and inflammation (6,7) in diabetes, possibly increasing the antioxidant defenses of the endothelium (8) and decreasing oxidative stress generation (6,7).

The combination of basal insulin and GLP-1 is now proposed for the management of type 2 diabetes (9), while a recent report has shown that GLP-1 can enhance the vasodilatory effect of insulin in subjects with the metabolic syndrome (10).

Based on this evidence, the therapeutic option of combining insulin and GLP-1 may have a significant impact on the cardiovascular risk profile of type 2 diabetic patients. The aim of this study was to evaluate the effect of adding GLP-1 to normal and increased insulin plasma levels, during both normo- and hyperglycemia, on several markers of endothelial function, inflammation, and oxidative stress.

## RESEARCH DESIGN AND METHODS

Two groups of 12 matched type 2 diabetic patients participated in the study. Baseline characteristics of the study groups are shown in Table 1. The study was approved by one of the ethics committees of our institutions, and written consent from the study subjects was obtained.

Ten patients were on diet alone, and the other 14 patients were on metformin, which was discontinued at least 4 weeks before the study. In none of the type 2 diabetic patients was retinopathy, nephropathy, or neuropathy present. Five patients had hypertension treated with an ACE inhibitor, which was withheld on the study days. None of the subjects were on statin or antioxidant supplementation.

Synthetic GLP-1 (7–36)amide was purchased from PolyPeptide Laboratories (Wolfenbuttel, Germany), and the same lot number was used in all studies.

**Table 1—Baseline characteristics of the type 2 diabetic patients**

| | Group 1 | Group 2 |
|---|---|---|
| Sex (male/female) | 9/3 | 8/4 |
| Age (years) | 53.2 ± 2.3 | 52.7 ± 1.3 |
| BMI (kg/m$^2$) | 26.8 ± 1.4 | 26.2 ± 2.1 |
| $HbA_{1c}$ (%) | 8.1 ± 0.5 | 8.0 ± 0.4 |
| $HbA_{1c}$ (mmol/mol) | 65 ± 3.2 | 65 ± 3.0 |
| Resting diastolic blood pressure (mmHg) | 79.3 ± 1.2 | 78.5 ± 1.5 |
| Resting systolic blood pressure (mmHg) | 115.2 ± 1.4 | 117.3 ± 1.6 |
| Total cholesterol (mmol/L) | 4.32 ± 0.3 | 4.22 ± 0.4 |
| Triglycerides (mmol/L) | 1.1 ± 0.4 | 1.3 ± 0.2 |
| HDL-C (mmol/L) | 1.4 ± 0.2 | 1.3 ± 0.3 |
| LDL-C (mmol/L) | 2.0 ± 0.2 | 2.1 ± 0.4 |
| FMD (%) | 6.3 ± 0.5 | 6.5 ± 0.3 |
| 8-iso-PGF2α (pg/mL) | 65.4 ± 3.2 | 69.4 ± 3.0 |
| Nitrotyrosine (μmol/L) | 0.77 ± 0.07 | 0.77 ± 0.04 |
| sICAM-1 (ng/mL) | 170.5 ± 12.5 | 176.5 ± 13.5 |
| IL-6 (pg/mL) | 220.35 ± 11.1 | 227.35 ± 11.3 |

Data are means ± SEM. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.

### Study Design

Subjects were admitted to the research center the evening before the experiment. All subjects received an evening meal and received a continuous low-dose infusion of insulin to normalize plasma glucose. The insulin infusion was adjusted overnight to maintain blood glucose between 4.4 and 7.2 mmol/L and stopped 2 h before the start of each experiment.

After a 12-h overnight fast, subjects were placed in a supine comfortable position with a room temperature between 20° and 24°C. Intravenous lines were inserted into a large antecubital vein of one arm for infusions and into a dorsal vein of the contralateral arm for blood sampling. Patency was preserved with a slow saline infusion (0.9% NaCl). The study began after the subjects had rested for 30 min.

Octreotide infusion (25 mg i.v. bolus followed by a 0.5 mg/min infusion, Longastatina; Italfarmaco, Milan, Italy) to block the release of endogenous insulin was started 5 min before the priming glucose pulse and was interrupted at the end of the clamp.

Group 1 had, in a randomized order and on different days, with at least a 1-week interval, two normoglycemic-normoinsulinemic clamps (11) (glucose infusion to maintain the concentration of 5 mmol/L), with or without GLP-1, and two normoglycemic-hyperinsulinemic clamps (insulin infusion 0.1 mU/kg/min and constant glucose infusion to maintain the concentration of 5 mmol/L) with or without GLP-1.

Group 2 had, in a randomized order and on different days, with at least a 1-week interval, two normoglycemic-normoinsulinemic clamps (glucose infusion to maintain the concentration of 15 mmol/L), with or without GLP-1, and two normoglycemic-hyperinsulinemic clamps (insulin infusion 0.1 mU/kg/min and constant glucose infusion to maintain the concentration of 15 mmol/L) with or without GLP-1.

All experiments lasted 2 h. GLP-1 was infused at a rate of 0.4 pmol · kg$^{-1}$ · min$^{-1}$, according to the methodology of Nauck et al. (12).

At baseline and at 1 and 2 h, plasma glucose, flow-mediated dilatation (FMD), nitrotyrosine, plasma 8-iso-prostaglandin F2α (8-iso-PGF2α), soluble intercellular adhesion molecule (sICAM-1), and interleukin (IL)-6 were measured.

### Biochemical and Clinical Measurements

Cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and plasma nitrotyrosine were measured according to the methodology of Ceriello et al. (13). Plasma glucose was measured by the glucose-oxidase method, $HbA_{1c}$ by high-performance liquid chromatography and insulin by microparticle enzyme immunoassay (Abbott Laboratories, Wiesbaden, Germany). 8-iso-PGF2α

(Cayman Chemical, Ann Arbor, MI), sICAM-1 (British Biotechnology, Abingdon, U.K.) and IL-6 (R&D Systems, Minneapolis, MN), were determined with commercially available kits.

Endothelial function at macrovascular level was evaluated by measuring the FMD of the brachial artery (6,7). The examination was carried out in a temperature and light-controlled room on subjects who were lying comfortably flat on a couch. Brachial arteries in this study were imaged with a standard ultrasound system (Vivid 7 ECHO machine; GE Vingmed System V) connected with a 12 MHz linear transducer probe. The ultrasound system was connected to a personal computer equipped with a frame grabber and artificial neural network wall detection software (vessel image analysis). Brachial artery FMD was determined using protocol similar to published studies (6,7).

At the end of the study each day, 250 μg sublingual glyceryl trinitrate was administered to assess endothelium-independent vasodilatation. The intraobserver variability for repeated measurements of resting arterial diameter was 0.02 ± 0.02 mm.

### Statistical Analysis

The sample size was selected according to previous studies (5–7,10). Data are expressed as means ± SE. The Kolmogorov-Smirnov algorithm was used to determine whether each variable had a normal distribution. Comparisons of baseline data among the groups were performed using unpaired Student *t* test or Mann-Whitney *U* test where indicated. The changes in variables during the tests were assessed by two-way ANOVA with repeated measures or Kolmogorov-Smirnov test where indicated. If differences reached statistical significance, post hoc analyses with two-tailed paired *t* test or Wilcoxon signed rank test for paired comparisons was used to assess differences at individual time periods in the study. Statistical significance was defined as $P < 0.05$.

## RESULTS

### Normoglycemic Clamps

In normoglycemic-normoinsulinemic conditions, all of the parameters remained unchanged (Fig. 1). During the normoglycemic-hyperinsulinemic clamp, FMD increased, while sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 decreased compared with normoglycemic-normoinsulinemic clamp (Fig. 1). GLP-1 infusion during the normoinsulinemic-normoinsulinemic clamp also increased FMD, and decreased sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 compared with normoglycemic-normoinsulinemic clamp (Fig. 1). The combination of hyperinsulinemia and GLP-1, in normoglycemia, was accompanied by a further FMD increase and sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 decrease (Fig. 1).

During the normoglycemic-normoinsulinemic clamp, the glucose infusion rate (GIR) was 3.2 ± 0.4 mg/kg/min and 3.5 ± 0.5 mg/kg/min adding GLP-1 (*P* = NS). During the normoglycemic-hyperinsulinemic clamp, GIR was 4.3 ± 0.6 mg/kg/min and 4.7 ± 0.5 mg/kg/min adding GLP-1 (*P* = NS).

### Hyperglycemic Clamps

During the hyperglycemic-normoinsulinemic clamp, FMD significantly decreased, while sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 significantly increased (Fig. 2). When hyperglycemia was accompanied by hyperinsulinemia or by the simultaneous infusion of GLP-1, all these phenomena were significantly attenuated: FMD decreased less, while sICAM-1, 8-iso-PGF2α, nitrotyrosine, and IL-6 were less increased (Fig. 2). The simultaneous presence of hyperinsulinemia and infusion of GLP-1 had even better effect (Fig. 2).

During the hyperglycemic-normoinsulinemic clamp, GIR was 4.5 ± 0.3 mg/kg/min and 4.4 ± 0.4 mg/kg/min adding GLP-1 (*P* = NS). During the hyperglycemic-hyperinsulinemic clamp, GIR was 6.1 ± 0.5 mg/kg/min and 6.4 ± 0.2 mg/kg/min adding GLP-1 (*P* = NS). Endothelial-independent vasodilatation was not affected in any of the experiments (data not shown).

## CONCLUSIONS

This study confirms that both insulin and GLP-1 have a vasodilatory, antiinflammatory, and antioxidant action (1–7). However, in this study, for the first time in type 2 diabetes, we show that when GLP-1 and insulin are simultaneously infused their vasodilatory, antiinflammatory, and antioxidant action is enhanced. It is worthy of interest that the effect of GLP-1 is almost equivalent to that of hyperinsulinemia and is present in both normo- and hyperglycemia.

Our data are partially in the same direction of the work of Tesauro et al. (10), which showed an enhancement of the vasodilatory action of insulin by GLP-1 in the metabolic syndrome. In the study of Tesauro et al. (10), GLP-1 was active in improving vasodilation only in the presence of hyperinsulinemia, while our study confirms that GLP-1 has a direct effect on endothelial function even in normoinsulinemia. In the same direction is the evidence that GLP-1 can induce endothelial vasodilation in normal subjects (14). Moreover, it is worthy of interest that in the study of Tesauro et al. (10), a lack of the effect of GLP-1 was reported in the obese subjects without the metabolic syndrome (10). A possible explanation is that people with the metabolic syndrome differ from other people, responding differently to GLP-1, as also suggested by Tesauro et al. (10).

It must also be underlined that in our study, GLP-1 was infused systemically, whereas in the study of Tesauro et al. (10) it has been given locally. Therefore, other potential factors might have been involved in the effects of GLP-1 on endothelial function.

Furthermore, our study shows that the antiinflammatory and antioxidant action of insulin and GLP-1 is also enhanced when they are acutely administered in type 2 diabetes. Evidence is cumulating showing that both insulin and GLP-1 alone have such characteristics (1–7); however, in our opinion, the possibility that when they are combined the single property of each of them is amplified is very interesting for the possible clinical implications.

The combination of insulin and GLP-1RA analogs is today receiving a lot of attention as a possible therapeutic choice for the management of type 2 diabetes (9). The evidence that this combination cannot only improve the metabolic control but also significantly impact on the cardiovascular risk profile of the diabetic patients is certainly of great interest. Of course, specific clinical trials are needed to confirm the usefulness of this therapeutic possibility.

The mechanism by which GLP-1 and insulin reciprocally enhance their properties remains speculative.

In diabetes, but not only in diabetes, it has been suggested that oxidative stress can favor the appearance of endothelial dysfunction and inflammation

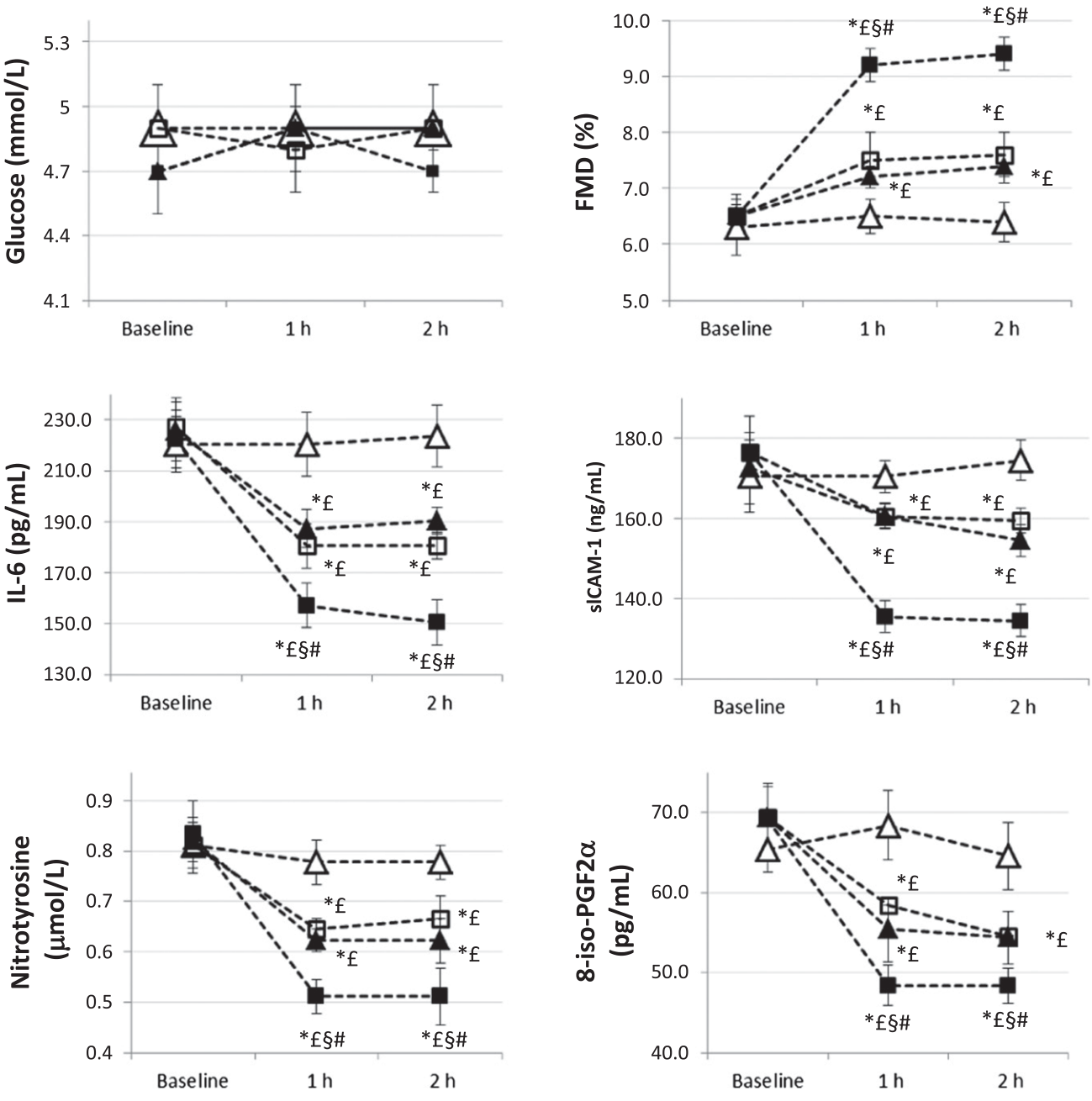


**Figure 1**—Changes in glycemia, FMD, IL-6, sICAM-1, nitrotyrosine, and 8-iso-PGF2α during normoglycemic-normoinsulinemic and normoglycemic-hyperinsulinemic clamps in type 2 diabetes ($n$ = 12). Glycemia, FMD, IL-6, sICAM-1, nitrotyrosine, and 8-iso-PGF2α changes during normoglycemic-normoinsulinemic clamp (△), normoglycemic-normoinsulinemic clamp plus GLP-1 (▲), normoglycemic-hyperinsulinemic clamp (□), and normoglycemic-hyperinsulinemic clamp plus GLP-1 (■). Data are means ± SEM. *$P < 0.01$ vs. basal. £$P < 0.05$ vs. normoglycemic-normoinsulinemic clamp. §$P < 0.05$ vs. normoglycemic-normoinsulinemic clamp plus GLP-1. #$P < 0.05$ vs. normoglycemic-hyperinsulinemic clamp.

(15,16). Both insulin and GLP-1 have an antioxidant property, which, however, follows different paths: insulin reduces free radical production (1), while GLP-1 increases the intracellular antioxidant defenses (8). Therefore, even though clearly this is not the only possible explanation, it could be hypothesized that when they are combined the final antioxidant effect is enhanced. This may result in a greater reduction of the oxidative stress, leading to a greater decrease of inflammation and to a better improvement of the endothelial dysfunction.

A possible interference of the somatostatin analog in our study could be hypothesized. Annamalai et al. (17) recently reported that chronic treatment (24 weeks) with a somatostatin analog, lanreotide, improved endothelial function in patients with newly diagnosed acromegaly. The study, therefore, reports a possible effect of somatostatin and its analogs on endothelial function when chronically administered. However, there are several studies reporting that somatostatin and its analogs do not have any effect on endothelial function when acutely administered (18–20). Therefore, a possible direct effect of somatostatin in our study can be convincingly excluded.

There was a slight difference in GIR between the experiments. This would

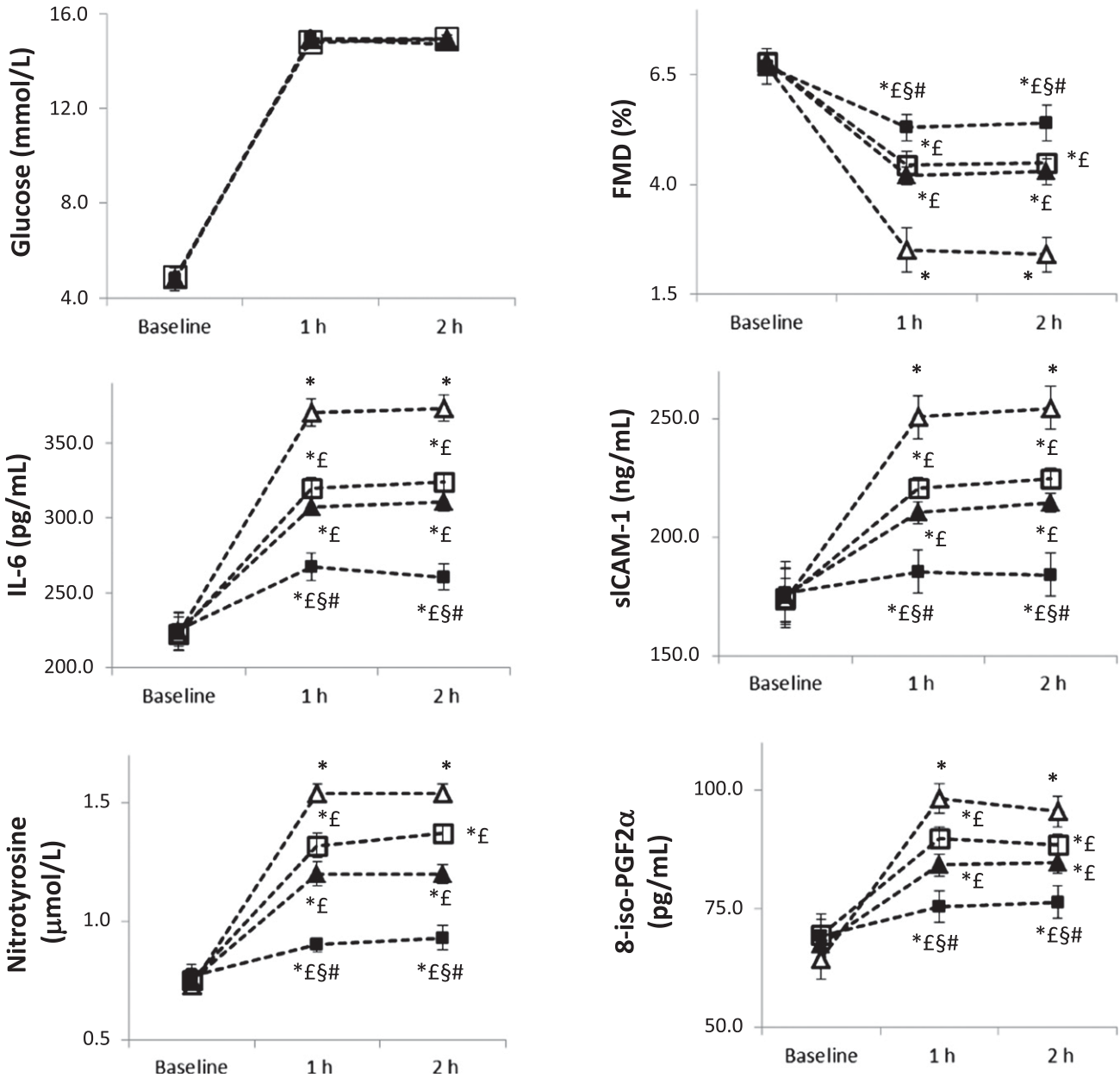


**Figure 2**—Changes in glycemia, FMD, IL-6, sICAM-1, nitrotyrosine, and 8-iso-PGF2α during hyperglycemic-normoinsulinemic and hyperglycemic-hyperinsulinemic clamps in type 2 diabetes ($n$ = 12). Glycemia, FMD, IL-6, sICAM-1, nitrotyrosine, and 8-iso-PGF2α changes during hyperglycemic-normoinsulinemic clamp (△), hyperglycemic-normoinsulinemic clamp plus GLP-1 (▲), hyperglycemic-hyperinsulinemic clamp (□), and hyperglycemic-hyperinsulinemic clamp plus GLP-1 (■). Data are mean ± SEM. *$P < 0.01$ vs. basal. £$P < 0.05$ vs. hyperglycemic-normoinsulinemic clamp. §$P < 0.05$ vs. hyperglycemic-normoinsulinemic clamp plus GLP-1. #$P < 0.05$ vs. hyperglycemic-hyperinsulinemic clamp.

mean a higher "glucose turnover" that in itself could be of relevance for endothelial dysfunction or its improvement. However, in our opinion, a higher glucose turnover is not supposed to ameliorate endothelial dysfunction. On the contrary, higher intracellular concentrations of glucose, according to the available literature (15), could mean increased intracellular oxidative stress. In this respect, we believe that our results are further strengthened.

In conclusion, this study shows, for the first time, that GLP-1 and insulin reciprocally enhance their vasodilatory, antioxidant, and antiinflammatory action, suggesting a further rationale for using their combination in the clinical practice.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.
**Author Contributions.** A.C. contributed to data research, discussion, writing the manuscript, and reviewing and editing the manuscript. A.N. and L.B. contributed to data research, discussion, and reviewing and editing the manuscript. S.C., K.E., R.T., M.R., and S.G. contributed to discussion and reviewing and editing the manuscript. L.L.S. and G.P. contributed to data research and discussion. A.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## References

1. Dandona P, Chaudhuri A, Ghanim H, Mohanty P. Insulin as an anti-inflammatory and antiatherogenic modulator. J Am Coll Cardiol 2009;53(Suppl.):S14–S20
2. Dandona P, Aljada A, Mohanty P, et al. Insulin inhibits intranuclear nuclear factor kappaB

and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 2001;86:3257–3265
3. Monnier L, Colette C, Mas E, et al. Regulation of oxidative stress by glycaemic control: evidence for an independent inhibitory effect of insulin therapy. Diabetologia 2010;53:562–571
4. Mudaliar S, Henry RR. Effects of incretin hormones on beta-cell mass and function, body weight, and hepatic and myocardial function. Am J Med 2010;123(Suppl.):S19–S27
5. Nyström T, Gutniak MK, Zhang Q, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. Am J Physiol Endocrinol Metab 2004;287:E1209–E1215
6. Ceriello A, Esposito K, Testa R, Bonfigli AR, Marra M, Giugliano D. The possible protective role of glucagon-like peptide 1 on endothelium during the meal and evidence for an "endothelial resistance" to glucagon-like peptide 1 in diabetes. Diabetes Care 2011;34:697–702
7. Ceriello A, Novials A, Ortega E, et al. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. Diabetes Care 2013;36:2346–2350
8. Oeseburg H, de Boer RA, Buikema H, van der Harst P, van Gilst WH, Silljé HH. Glucagon-like peptide 1 prevents reactive oxygen species-induced endothelial cell senescence through the activation of protein kinase A. Arterioscler Thromb Vasc Biol 2010;30:1407–1414
9. van der Klauw MM, Wolffenbuttel BH. The combination of insulin and GLP-1 analogues in the treatment of type 2 diabetes. Neth J Med 2012;70:436–443
10. Tesauro M, Schinzari F, Adamo A, et al. Effects of GLP-1 on forearm vasodilator function and glucose disposal during hyperinsulinemia in the metabolic syndrome. Diabetes Care 2013;36:683–689
11. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223
12. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993;91:301–307
13. Ceriello A, Mercuri F, Quagliaro L, et al. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. Diabetologia 2001;44:834–838
14. Okerson T, Chilton RJ. The cardiovascular effects of GLP-1 receptor agonists. Cardiovasc Ther 2012;30:e146–e155
15. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058–1070
16. Rashid K, Sinha K, Sil PC. An update on oxidative stress-mediated organ pathophysiology. Food Chem Toxicol 2013;62:584–600
17. Annamalai AK, Webb A, Kandasamy N, et al. A comprehensive study of clinical, biochemical, radiological, vascular, cardiac, and sleep parameters in an unselected cohort of patients with acromegaly undergoing presurgical somatostatin receptor ligand therapy. J Clin Endocrinol Metab 2013;98:1040–1050
18. Marfella R, Verrazzo G, Acampora R, et al. Glutathione reverses systemic hemodynamic changes induced by acute hyperglycemia in healthy subjects. Am J Physiol 1995;268:E1167–E1173
19. Marfella R, Nappo F, De Angelis L, Siniscalchi M, Rossi F, Giugliano D. The effect of acute hyperglycaemia on QTc duration in healthy man. Diabetologia 2000;43:571–575
20. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002;106:2067–2072