Acute effects of hyperinsulinemia and hyperglycemia on vascular inflammatory biomarkers and endothelial function in overweight and obese humans

Jennifer M. Perkins,1 Nino G. Joy,2 Donna B. Tate,2 and Stephen N. Davis2

1Duke University, Chapel Hill, North Carolina; and 2Department of Medicine, University of Maryland, Baltimore, Baltimore, Maryland

Submitted 10 February 2015; accepted in final form 19 May 2015

Perkins JM, Joy NG, Tate DB, Davis SN. Acute effects of hyperinsulinemia and hyperglycemia on vascular inflammatory biomarkers and endothelial function in overweight and obese humans. Am J Physiol Endocrinol Metab 309: E168–E176, 2015. First published May 26, 2015; doi:10.1152/ajpendo.00064.2015.—We investigated the separate and combined effects of hyperglycemia and hyperinsulinemia on markers of endothelial function, proinflammatory and proatherothrombotic responses in overweight/obese nondiabetic humans. Twenty-two individuals (13 F/9 M, BMI 30.1 ± 4.1 kg/m2) were studied during four randomized, single-blind protocols. The pancreatic clamp technique was combined with 4-h glucose clamps consisting of either 1) euinsulinemia-euglycemia, 2) euinsulinemia-hyperglycemia, 3) hyperinsulinemia-hyperglycemia, or 4) hyperinsulinemia-euglycemia. Insulin levels were higher (998 ± 66 vs. 194 ± 22 pmol/l) during hyperinsulinemia compared with euinsulinemia. Glucose levels were 11.1 mmol/l during hyperinsulinemia compared with 5.1 ± 0.1 mmol/l during euglycemia. VCAM, ICAM, P-selectin, E-selectin, IL-6, adiponectin, and PAI-1 responses were all increased (P < 0.01-0.0001), and endothelial function was decreased (P < 0.0005) during euinsulinemia-hyperglycemia compared with other protocols. The prevalence of hyperinsulinemia in the presence of hyperglycemia prevented the increase in proinflammatory and proatherothrombotic markers while also normalizing vascular endothelial function. We conclude that 4 h of moderate hyperglycemia can result in increases of proinflammatory markers (ICAM, VCAM, IL-6, E-selectin), platelet activation (P-selectin), reduced fibrinolytic balance (increased PAI-1), and disordered endothelial function in a group of obese and overweight individuals. Hyperinsulinemia prevents the actions of moderate hyperglycemia to reduce endothelial function and increase proinflammatory and proatherothrombotic markers.

hyperglycemia; endothelial function; hyperinsulinemia; inflammation

There are accumulating data that hyperglycemia can induce atherothrombotic mechanisms, reduce fibrinolytic balance, and impair endothelial function in both nondiabetic and diabetic individuals (9, 15–17, 44). The in vivo vascular biological effects of insulin are controversial, with studies reporting either deleterious or beneficial effects on endothelial function and atherothrombotic balance (24, 28, 32, 52).

THE PREVALENCE OF OBESITY has increased dramatically worldwide over the last 30 years (14). Obesity is now an independent risk factor for type 2 diabetes, cancer, and cardiovascular disease (12, 29, 36, 38, 50). Recent data from Schmidt et al. (51) report that obese individuals have an increased risk of venothrombotic disease in addition to myocardial infarction and/or stroke. Previous studies have demonstrated that obesity is a proinflammatory state (30, 47, 55). However, the acute specific effects of insulin and hyperglycemia, either alone or in combination, on endothelial function and proinflammatory and proatherothrombotic markers in obese and overweight individuals remain largely unknown.

Address for reprint requests and other correspondence: S. N. Davis, Dept. of Medicine, Univ. of Maryland, Baltimore, N3W42, 22 S. Greene St., Baltimore, MD 21201 (e-mail: sdavis@medicine.umd.edu).
RESEARCH DESIGN AND METHODS

Subjects. Twenty-two adult volunteers (13 F/9 M), age 41 ± 3 yr, BMI 30.1 ± 1 kg/m², HbA₁c 5.5 ± 0.1%, fat 29 ± 2%, were studied. None of the subjects smoked, received anticoagulants, clopidogrel, statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or oral insulin sensitizers (metformin, thiazolidinediones). Subjects over age 40 yr were screened for silent ischemia with an electrocardiogram. Each subject had normal blood count, plasma electrolytes, and liver and renal function and no evidence of either impaired fasting glucose or overt diabetes mellitus. Participants were randomized to undergo one of four protocols (Fig. 1), which consisted of an initial pancreatic clamp (8) followed by a single-step 4-h glucose clamp. The intention was for subjects to complete all four protocols. However, withdrawals occurred for the following reasons: 1) time schedule conflicts, 2) intolerance to nitroglycerin, or 3) inability to maintain iv access. Additional subjects were recruited to replace the individuals that withdrew and complete the remaining experiments in the randomized four-protocol block. Thus, seven participants completed all four protocols; four participants completed three protocols; six participants completed two protocols; and five participants completed one study protocol. All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board.

Experimental design. All individuals refrained from caffeine, exercise, and alcohol 24 h prior to the study. Participants were instructed to not use aspirin, NSAIDs, or COX 2 inhibitors for 3 days prior to a study. Subjects were admitted to the General Clinical Research Center the night prior to the study at 5:00 PM. After an overnight 10-h fast, two intravenous cannulae were inserted under 1% Lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand of the nondominant arm. This hand was placed in a heated box (55–60°C) during the study so that arterialized blood could be obtained (1). The other cannula was placed in the ipsilateral arm for infusions.

Pancreatic clamp. The somatostatin analog octreotide was infused at 30 ng·kg⁻¹·min⁻¹ to inhibit endogenous insulin, glucagon, and growth hormone secretion. Basal replacement amounts of insulin (1.8 pmol·kg⁻¹·min⁻¹), human growth hormone (3 ng·kg⁻¹·min⁻¹), and a variable basal amount of glucagon were infused throughout each experiment (until time 240 min). Glucagon was adjusted as needed to maintain euglycemia of ~5 mmol/l. Arterialized venous blood was sampled every 10–15 min to monitor plasma glucose.

Glucose clamp studies. After stable euglycemia was established during the pancreatic clamp, 4-h single-step glucose clamps at differing glycemia and hyperinsulinemia (euinsulinemia-euglycemia n = 14; euinsulinemia-hyperglycemia n = 15; hyperglycemia-euinsulinemia n = 14; hyperinsulinemia-euglycemia n = 14) were performed (Fig. 1). At time ~120 min, a constant (1.8 pmol·kg⁻¹·min⁻¹) infusion of insulin (Human Regular Insulin; Eli Lilly, Indianapolis, IN) was started via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA). At time 0 min, the insulin infusion was either maintained at 1.8 pmol·kg⁻¹·min⁻¹ or increased to 9 pmol·kg⁻¹·min⁻¹ and continued until 240 min. Ends of pancreatic clamp glucagon and growth hormone infusion rates were maintained unchanged during the 240 min glucose clamp procedures. Glucose targets of (5 mmol/l, 90 mg/dl), or (11.1 mmol/l, 200 mg/dl), depending on protocol, were achieved using a modification of the glucose clamp technique (23). During the clamp period, plasma glucose was measured every 5 min, and a 20% dextrose infusion was adjusted so that plasma glucose levels were held constant. Potassium chloride (20 mmol/l) was infused during hyperinsulinemic clamp studies to reduce insulin-induced hypokalemia.

Analytic methods. The collection and processing of blood samples have been described elsewhere (18). Plasma glucose concentrations were measured in triplicate every 5 min using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Blood for insulin, catecholamines (epinephrine and norepinephrine), nonesterified fatty acids (NEFA), glucagon, cortisol, growth hormone, and C-peptide was drawn every 30 min during the experimental period. Insulin was measured as previously described (58) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. Two modifications to the procedure for catecholamine determination were made: 1) a five-point rather than a one-point calibration curve was used; and 2) baseline levels were determined at the start of each protocol. Plasma epinephrine and norepinephrine were measured by HPLC (13) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. Insulin was measured as previously described (58) with an interassay CV of 9%. Catecholamines were determined by HPLC (13) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. Two modifications to the procedure for catecholamine determination were made: 1) a five-point rather than a one-point calibration curve was used; and 2) baseline levels were determined at the start of each protocol.
RESULTS

Glucose and insulin. Plasma glucose was maintained at equivalent levels during euglycemic clamps (5.1 ± 0.1 vs. 5.0 ± 0.1 mmol/l) and were equivalent during all hyperglycemic clamp protocols (11.2 ± 0.1 vs. 11.1 ± 0.2 mmol/l) (Fig. 2). Insulin levels during euinsulinemia (194 ± 22 vs. 210 ± 18 pmol/l) and hyperinsulinemia (998 ± 66 vs. 988 ± 114 pmol/l) groups were similar during the respective clamp studies (Fig. 2). The glucose infusion rates (µmol·kg−1·min−1) required to maintain euglycemia were 11.8 ± 2.3 during the euinsulinemic-euglycemic control study and 41.3 ± 3.3 during the hyperinsulinemic-euglycemic clamps. Glucose infusion rates during the euinsulinemic-hyperglycemic and hyperinsulinemic-hyperglycemic clamps were 28.9 ± 4.6 and 70.5 ± 6.1 µmol·kg−1·min−1, respectively.

Neuroendocrine counterregulatory hormones. Baseline (time 0) epinephrine, norepinephrine, glucagon, growth hormone, and cortisol levels were similar at the start of all glucose clamp studies. Norepinephrine increased similarly during all protocols. Other neuroendocrine counterregulatory hormones remained similar to baseline in all studies (Table 1).

Intermediary metabolism. Blood NEFA and triglyceride levels were similar at the start of all protocols (time 0) and fell by a similar and a significant amount during hyperglycemic and hyperinsulinemic clamps (P < 0.008; Table 1).

Atherogenic vascular adhesion molecules. Baseline values of VCAM, ICAM, and E-selectin were similar at baseline (time 0) during all four protocols (Table 2). VCAM, ICAM, and E-selectin responses increased (P < 0.0001) during euinsulinemia-hyperglycemia but fell (P < 0.0001) compared with baseline during the other protocols (Table 2 and Fig. 3). The responses of VCAM, ICAM, and E-selectin during euinsulinemia-hyperglycemia were significantly increased (P < 0.0001) compared with the other three protocols when compared as
either time course or as baseline to end of clamp responses (Fig. 3). VCAM, ICAM and E-selectin fell from baseline ($P < 0.0001$) during all hyperinsulinemic protocols.

**Platelet activation and fibrinolytic balance.** Baseline P-selectin, tPA, and PAI-1 were similar at the start of the four glucose clamp protocols (Table 2). P-selectin and PAI-1 responses and levels were higher ($P < 0.02$-$0.0001$) during euinsulinemia-hyperglycemia and were significantly increased ($P < 0.0001$) compared with the other three protocols (Table 2 and Fig. 3). PAI-1 and P-selectin levels fell from baseline ($P < 0.02$-$0.0001$) during the hyperinsulinemic protocols. There was no difference in tPA during any protocol (Table 2).

**Adiponectin and IL-6.** Adiponectin responses increased ($P < 0.0003$) during euinsulinemia-hyperglycemia compared with falls during the other three protocols (Table 2 and Fig. 3). IL-6 increased significantly ($P < 0.02$) from baseline during euinsulinemia-hyperglycemia (Table 2), and responses were increased ($P < 0.02$) compared with all other protocols (Table 2 and Fig. 3).

**Endothelial function.** Brachial artery diameter was similar at the start of all endogenously NO-mediated vasodilation studies (4.4 ± 0.1 and 4.5 ± 0.1 mm) at baseline and end of clamp measurements. The increase in flow-mediated dilation during endogenous NO stimulation was significantly blunted during euinsulinemia-hyperglycemia compared with all other protocols ($P = 0.0005$; Fig. 4). Similar to the above proinflammatory and proatherothrombotic biomarkers, this effect was reversed during hyperinsulinemic hyperglycemia and euglycemic protocols (Fig. 4).

There were similar increases in vasodilation among all protocols during exogenous NO donation with nitroglycerin administration.

There was no correlation between changes in baseline flow-mediated dilation during endogenous NO stimulation and insulin action (glucose infusion rates) as measured during the euglycemic hyperinsulinemic clamps ($r = 0.15, P = 0.61$). The CV of the baseline FMD measurements was 4.5 ± 0.7% with a range of 1 to 10% per individual.

**DISCUSSION**

In the present study, we have demonstrated, by incorporating the pancreatic clamp technique to allow determination of the independent effects of hyperglycemia and hyperinsulinemia, that 4 h of moderate hyperglycemia (11.1 mmol/l) produces acute impairment of endothelial function and increases proatherothrombotic biomarkers in overweight and/or obese individuals. Our study extends previous reports demonstrating that hyperglycemia can simultaneously impair multiple components of vascular function (9, 15–17, 44). The proatherogenic adhesion molecules VCAM-1, E-selectin, and ICAM-1 were increased acutely by modest hyperglycemia. P-selectin, a marker of platelet activation and endothelial dysfunction (27), also increased during hyperglycemia. Furthermore, hyperglycemia impaired fibrinolytic balance by limiting the usual diurnal fall in PAI-1. This created a condition of relatively increased levels of PAI-1 with unchanged systemic values of tPA. Hyperglycemia also blunted endogenous NO-mediated endothelial function, which can be a predictor of future car-
diovascular events (6, 46, 49). Last, the significant increases of IL-6 and adiponectin demonstrate that acute hyperglycemia also results in activation of powerful systemic cytokines. The present study has also demonstrated that acute high physiological levels of insulin are able to prevent the proinflammatory and proatherothrombotic effects of moderate hyperglycemia. The effects of insulin on in vivo integrated vascular biological function are controversial. Large cross-sectional studies in nondiabetics or individuals with impaired glucose tolerance have linked hyperinsulinemia with an increased risk of cardiovascular events (9, 28). Complicating interpretation of these studies is that hyperinsulinemia may be resultant on an underlying putative mechanism such as insulin resistance. Thus, it is unclear what role insulin per se may be playing in the occurrence of these cardiovascular events. Somewhat surprisingly, there have been relatively few in vivo human studies performed investigating the integrated effects of insulin on inflammation and atherothrombotic balance. The majority, but not all, report beneficial effects of insulin during experimental euglycemic studies, even under significant clinical conditions such as myocardial infarction (22). However, data addressing the integrated vascular biological effect of hyperinsulinemia during conditions of hyperglycemia are very scarce. Only two studies appear to have addressed this topic, with somewhat differing results (53, 59). Williams et al. (59) reported that modest increases of insulin could moderate the deleterious effects of hyperglycemia on endothelial function, whereas Stegenga et al. (53) reported that hyperinsulinemia impairs fibrinolysis in six healthy, young males. Recently, Ceriello et al. (15) have also demonstrated that hyperglycemia of 15 mmol/l produces proinflammatory changes and endothelial dysfunction in a group of lean, young type 1 diabetic individuals, thus reinforcing that hyperglycemia per se in the presence of only basal insulin levels can result in changes of a wide range of vascular biomarkers. However, no study has addressed the vascular biological effects of insulin during hyperglycemia in overweight/obese individuals. Our results clearly demonstrate that hyperinsulinemia can prevent the wide spectrum of deleterious effects caused by hyperglycemia on proatherothrombotic balance and endothelial function. Hyperinsulinemia in the presence of hyperglycemia normalized the responses of adhesion molecules (ICAM, VCAM, E-selectin),

Fig. 3. Timeline responses from baseline of adiponectin, ICAM, P-selectin, IL-6, E-selectin, PAI-1, and VCAM during euglycemic (5.0 mmol/l) and hyperglycemic (11.1 mmol/l) clamps in the presence of octreotide and insulin at 1.8 or 9 pmol·kg⁻¹·min⁻¹ in overnight-fasted obese/overweight humans. *Response during euinsulinemic hyperglycemia is significantly increased (P < 0.01-0.0001) vs. responses in the other groups. †Response is significantly decreased (P < 0.02-0.0001) vs. baseline. #Response is significantly increased (P < 0.02-0.0001) vs. baseline.
platelet activation (P-selectin), fibrinolytic balance (PAI-1), inflammation (IL-6, adiponectin), and endothelial function created by hyperglycemia. In fact, the responses of the above vascular biological biomarkers during hypersulinemic hyperglycemia were no different compared with the time control of euglycemic euinsulinemia or the hyperinsulinemic-euglycemic control. These latter two control protocols demonstrate that 1) acute hyperinsulinemia has no deleterious effects on atherothrombotic and fibrinolytic balance under euglycemic conditions; 2) acute hyperinsulinemia has widespread anti-inflammatory effects by reducing proatherothrombotic markers and improving endothelial function; and 3) it is acute hyperglycemia rather than hyperinsulinemia that has detrimental vascular biological effects.

This study also provides some mechanistic insights regarding the protective effects of insulin on vascular physiology. Hyperglycemia and hyperinsulinemia appear to be exerting opposite physiological effects by direct actions on vascular smooth muscle. Our endothelial function studies clearly demonstrate that hyperglycemia is acting via an endogenous NO mechanism to impair endothelial function, which can be reversed by hyperinsulinemia and prevented by an exogenous NO donor. IL-6 was significantly increased by hyperglycemia, but this elevation was prevented by hyperinsulinemia. Hyperglycemia as well as insulin resistance are known to activate NF-κB, which stimulates production of the proinflammatory cytokine IL-6, whereas insulin has been demonstrated to inhibit NF-κB activity (21). Adiponectin also increased during isolated hyperglycemia. Adiponectin is known to have insulin-sensitizing and antiatherogenic properties and thus may be acting in a “counterregulatory” manner to oppose the proinflammatory properties of hyperglycemia. Hyperglycemia also had a profound effect on suppressing free fatty acids (FFA). In fact, FFAs were similarly suppressed during the two hyperinsulinemic protocols compared with isolated hyperglycemia. This demonstrates that in this study increases in FFA cannot be implicated as a mechanism for the deleterious effects of hyperglycemia per se on the vascular endothelium. This point is worth noting as there are several studies demonstrating that high levels of FFAs (and triglycerides) can also result in adverse vascular biological effects (20, 56). Catecholamines (epinephrine, norepinephrine), cortisol, glucagon, and growth hormone were similar among all protocols. All of the above endocrine hormones have been demonstrated to have significant effects on the vascular endothelium and fibrinolytic balance (3, 19, 25, 34, 41, 57). The use of the pancreatic clamp allowed equivalent replacement of growth hormone during all protocols. This element of the experimental design is distinct to this present study. Other studies using the pancreatic clamp have not replaced growth hormone, and hyperglycemia is also known to suppress the hormone. Both would result in a state of growth hormone deficiency. This may have some relevance, as growth hormone deficiency has recently been reported to increase PAI-1 levels (39).

The PAI-1 gene is known to have glucose and insulin response elements (11). Although there is general agreement that hyperglycemia can increase PAI-1 levels, the effects of insulin on the molecule are more contentious (3). Our present study also provides new information regarding the independent effects of hyperglycemia and hyperinsulinemia on proinflammatory and proatherothrombotic biomarkers in obese and overweight humans. These individuals have an accelerated incidence of coronary artery disease, type 2 diabetes, and cancer, all of which have important inflammatory components. End of clamp or time course responses demonstrate that isolated hyperglycemia on a background of euinsulinemia increased a broad spectrum of proinflammatory and proatherothrombotic biomarkers; and antiatherogenic properties and thus may be acting in a “counterregulatory” manner to oppose the proinflammatory effects by reducing proatherothrombotic markers and improving endothelial function; and 3) it is acute hyperglycemia rather than hyperinsulinemia that has detrimental vascular biological effects.

This study also provides some mechanistic insights regarding the protective effects of insulin on vascular physiology. Hyperglycemia and hyperinsulinemia appear to be exerting opposite physiological effects by direct actions on vascular smooth muscle. Our endothelial function studies clearly demonstrate that hyperglycemia is acting via an endogenous NO mechanism to impair endothelial function, which can be reversed by hyperinsulinemia and prevented by an exogenous NO donor. IL-6 was significantly increased by hyperglycemia, but this elevation was prevented by hyperinsulinemia. Hyperglycemia as well as insulin resistance are known to activate NF-κB, which stimulates production of the proinflammatory cytokine IL-6, whereas insulin has been demonstrated to inhibit NF-κB activity (21). Adiponectin also increased during isolated hyperglycemia. Adiponectin is known to have insulin-sensitizing and antiatherogenic properties and thus may be acting in a “counterregulatory” manner to oppose the proinflammatory properties of hyperglycemia. Hyperglycemia also had a profound effect on suppressing free fatty acids (FFA). In fact, FFAs were similarly suppressed during the two hyperinsulinemic protocols compared with isolated hyperglycemia. This demonstrates that in this study increases in FFA cannot be implicated as a mechanism for the deleterious effects of hyperglycemia per se on the vascular endothelium. This point is worth noting as there are several studies demonstrating that high levels of FFAs (and triglycerides) can also result in adverse vascular biological effects (20, 56). Catecholamines (epinephrine, norepinephrine), cortisol, glucagon, and growth hormone were similar among all protocols. All of the above endocrine hormones have been demonstrated to have significant effects on the vascular endothelium and fibrinolytic balance (3, 19, 25, 34, 41, 57). The use of the pancreatic clamp allowed equivalent replacement of growth hormone during all protocols. This element of the experimental design is distinct to this present study. Other studies using the pancreatic clamp have not replaced growth hormone, and hyperglycemia is also known to suppress the hormone. Both would result in a state of growth hormone deficiency. This may have some relevance, as growth hormone deficiency has recently been reported to increase PAI-1 levels (39).

The PAI-1 gene is known to have glucose and insulin response elements (11). Although there is general agreement that hyperglycemia can increase PAI-1 levels, the effects of insulin on the molecule are more contentious (3). Our present study also provides new information regarding the independent effects of hyperglycemia and hyperinsulinemia on proinflammatory and proatherothrombotic biomarkers in obese and overweight humans. These individuals have an accelerated incidence of coronary artery disease, type 2 diabetes, and cancer, all of which have important inflammatory components. End of clamp or time course responses demonstrate that isolated hyperglycemia on a background of euinsulinemia increased a broad spectrum of proinflammatory and proatherothrombotic biomarkers; and antiatherogenic properties and thus may be acting in a “counterregulatory” manner to oppose the proinflammatory effects by reducing proatherothrombotic markers and improving endothelial function; and 3) it is acute hyperglycemia rather than hyperinsulinemia that has detrimental vascular biological effects.
proatherothrombotic biomarkers (including platelet aggregation and reducing fibrinolytic balance). Thus, hyperglycemia can potentially amplify an existing proinflammatory and procoagulant state in obese and/or overweight individuals. Hyperinsulinemia, on the other hand, reversed the proinflammatory and proatherothrombotic effects of hyperglycemia. Furthermore, hyperinsulinemia suppressed proinflammatory and proatherothrombotic biomarkers on a background of euglycemia.

Our study utilized the pancreatic clamp as this is the only methodology that allows breaking normal physiological insulin and glucose feedback loops in nondiabetic humans. Octreotide was infused in all studies to allow us to take control of the endocrine pancreas and growth hormone secretion. Octreotide has been reported to have anti-inflammatory effects and thus may have suppressed some or all of our biomarker responses (42). In addition, there was a mild increase in basal insulin levels (90–162 pmol/l), which in the presence of eu glycemia would also have suppressed FFA, triglycerides, and potentially atherothrombotic biomarkers. Furthermore, circadian rhythms would have resulted in decreases in PAI-1 during the duration of our studies. However, this would not have affected our qualitative study conclusions as all results were compared with the time and pancreatic clamp control of euinsulinemic eu glycemia. We selected a moderate hyperglycemic stimulus of 11.1 mmol/l (200 mg/dl) and cannot comment on whether a higher glycemc target for a longer duration would have produced a greater proinflammatory and/or proatherothrombotic biomarker response.

In summary, the present study has demonstrated, using pancreatic and glucose clamps to control glucose and insulin levels, that hyperglycemia results in significant increases in atherogenic adhesion molecules (ICAM, VCAM, E-selectin), platelet activation (P-selectin), inflammatory cytokines (IL-6), reduced fibrinolytic balance (increased PAI-1), and impaired endothelial flow-mediated dilation. High physiological levels of insulin (albeit higher than levels obtained during insulin replacement therapy in diabetic individuals) prevented the deleterious, proinflammatory, procoagulant, and prothrombotic effects of hyperglycemia. Hyperinsulinemia also reversed the effects of hyperglycemia to impair endogenous NO mediated endothelial function.

In conclusion, this study has identified the specific effects of acute moderate hyperglycemia and hyperinsulinemia on in vivo vascular biomarkers in obese and overweight humans. Plasma glucose levels of 11.1 mmol/l produced a wide spectrum of adverse vascular pathophysiological responses. Concurrent hyperinsulinemia prevented these effects and protected endothelial function and atherothrombotic and fibrinolytic balance against hyperglycemia. Thus, 1) hyperinsulinemia was able to prevent an amplification of the proinflammatory and procoagulant state present in obese and overweight individuals, and 2) an important acute vascular biological action of insulin may be to protect the vasculature against the harmful effects of hyperglycemia.

ACKNOWLEDGMENTS

We thank Wanda Sneed, Eric Allen, the Vanderbilt Hormone Assay Core laboratory, Joe Covington, Jesse Gilliam, Antoinette Richardson, Cheryl Williams and Lisa Young for their excellent technical assistance. We also thank the nursing staff of the Vanderbilt Clinical Research Center for their excellent care. We also acknowledge the expert technical advice and help of Hegen Chen PhD in the analysis of this data.

GRANTS

This work was supported by the following NIH grants: P50 HL-081009 NIH/NIHBLI, RO1 DK-069803 NIH/NIIDDK, PO1 HL-056693 NIH/NHLBI, Vanderbilt Diabetes Research and Training grant (DRTC) P60 DK-020593 NIH/NIIDDK, Vanderbilt General Clinical Research Center TL1 TR-00447 NIH/NCRR. We also thank Takeda Pharmaceuticals for a fellowship award to Nino G. Joy.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

15. Cierello A, Novials A, Ortega E, Canivell S, LaSala L, Pujadas G, Esposito K, Giugliano D, Genovese S. Glucagon-like peptide reduces endothelial dysfunction, inflammation, and oxidative stress induced by


