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Comparative effects of acute Hypoglycemia and Hyperglycemia on Pro-Atherothrombotic Biomarkers and Endothelial Function in Non-Diabetic Humans

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Abstract

Background—The comparative effects of acute moderate hyperglycemia and hypoglycemia on in-vivo endothelial function together with pro-inflammatory and pro-atherothrombotic responses in healthy individuals have not been determined.

Methods—To investigate this question 45 healthy subjects were compared during glucose clamp studies consisting of euinsulinemic hyperglycemia and hyperinsulinemic hyperglycemia (plasma glucose 11.1 mmol/L, both with pancreatic clamps) and hyperinsulinemic euglycemia and hyperinsulinemic hypoglycemia (plasma glucose 5.1 and 2.9 mmol/L, respectively). Two D doppler ultrasound was used to determine brachial artery endothelial function.

Results—Insulin levels during euinsulinemia hyperglycemia were 194±23 and (850±49–988±114) pmol/L during all hyperinsulinemic protocols. Responses of VCAM-1, ICAM-1, E-Selectin, P-selectin, PAI-1, and IL-6 were increased ($p<0.05$-0.0001) during euinsulinemic hyperglycemia or hypoglycemia as compared to hyperinsulinemic euglycemia or hyperinsulinemic hyperglycemia. PAI-1 was increased ($p<0.04$) during hypoglycemia as compared to euinsulinemic hyperglycemia, TNF-α responses were also increased during hypoglycemia as compared to hyperinsulinemic euglycemia or hyperinsulinemic hyperglycemia ($p<0.05$). In vivo endothelial function was similarly blunted by acute moderate hyperglycemia or hypoglycemia.

Conclusion—In summary, acute moderate hypoglycemia and euinsulinemic hyperglycemia can result in similar endothelial dysfunction and pro-atherothrombotic responses. Fibrinolytic balance was reduced by a greater extent by hypoglycemia as compared to moderate hyperglycemia. Acutely, hyperinsulinemia can prevent the acute pro-atherothrombotic and pro-inflammatory effects of moderate hyperglycemia but not hypoglycemia.

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Keywords

hyperglycemia; hypoglycemia; hyperinsulinemia; endothelial function; inflammation

1. INTRODUCTION

Recent data has suggested an association between severe hypoglycemia and increased serious cardiovascular morbidity and mortality (18, 21, 36, 38). Moreover, several studies have demonstrated or reported that acute hyperglycemia (5, 6, 9, 23, 27) or hypoglycemia (7, 25, 32, 41) can result in increased atherothrombotic mechanisms, reduced fibrinolytic balance, and impaired endothelial function in both nondiabetic and diabetic individuals (6, 10, 25, 32, 37). Despite recent data reporting the acute effects of hyperglycemia and hypoglycemia on pro-atherothrombotic responses, only one study has compared their relative effects in humans (9). Ceriello et al., studying effects of GLP-1 in individuals with type 1 DM, suggested that both hyperglycemia and hypoglycemia could be considered equivalent pro-atherosclerotic risk factors (9). However, there are no data available directly comparing the acute pro-inflammatory and pro-atherothrombotic effects of hypoglycemia and hyperglycemia in non-diabetic individuals. Therefore in this present report we have reanalyzed two of our recent articles (32, 40) in order to compare the acute effects of moderate hyperglycemia (11.1 mmol/L) with and without hyperinsulinemia and moderate hypoglycemia (2.9 mmol/L) on pro-inflammatory, pro-atherothrombotic and pro-coagulant biomarker responses in healthy individuals. The pancreatic clamp technique and various glucose clamp methodologies (euglycemic, hyperglycemic and hypoglycemic) (40) were combined so that the acute independent effects of glycemia and hyperinsulinemia could be determined.

2. METHODS

2.1 Subjects

45 adult volunteers (21M/24F, 38±3yrs, BMI 29±2kg/m^2 (23–36kg/m^2), HbA1C 5.2±0.2%), were studied. None of the participants smoked, received anticoagulants, clopidogrel, statins, angiotensin converting enzyme inhibitors, angiotensin receptor blockers or oral insulin sensitizers (metformin, thiazolidinediones). Each participant had normal blood count, plasma electrolytes, liver and renal function and no evidence of either impaired fasting glucose or overt diabetes mellitus. Study volunteers participated in four single blind one day protocols consisting of: 1) euinsulinemic/hyperglycemia (n=15) 2) hyperinsulinemic euglycemia (n=16) 3) hyperinsulinemic hyperglycemia (n=14) and 4) hyperinsulinemic hypoglycemia (n=16) Protocols 1 and 3 consisted of an initial pancreatic clamp (40) combined with a single step 4 hour hyperglycemic (11.1 mmol/L) clamp with either euinsulinemia or hyperinsulinemia. Protocol 2 and 4 consisted of a one step hyperinsulinemic euglycemic clamp (5 mmol/L) or hyperinsulinemic hypoglycemic clamp (2.9 mmol/L, both without pancreatic clamps). Data from this manuscript have been included in 2 separate reports (32, 40) examining 1) the independent effects of insulin and hyperglycemia on pro-inflammatory responses (40) and 2) effects of antecedent hypoglycemia on pro-inflammatory and pro-atherothrombotic responses in healthy humans

(32). All study participants gave written informed consent. Studies were approved by the Vanderbilt University and University of Maryland Human Subjects Institutional Review Board.

2.2 Experimental Design

All individuals refrained from caffeine, exercise, and alcohol for 24 hours prior to the study. Participants were instructed to not use aspirin, NSAIDs or COX 2 inhibitors for three days prior to a study. Subjects were admitted to the General Clinical Research Center (GCRC) the night prior to the study at 5pm. After an overnight 10 hr 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 non-dominant 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 also placed in the non-dominant arm for infusions.

2.3 Pancreatic Clamp and Glucose Clamp Studies

At time −120 min the somatostatin analogue, octreotide, was infused at 30 ng/kg/min to inhibit endogenous insulin, glucagon, and growth hormone secretion. Basal replacement amounts of human regular insulin (1.8 pmol/kg/min) (Lilly USA, Indianapolis, IN), human growth hormone (3 ng/kg/min) (Pharmacia & UpJohn, New York, NY) and a variable basal amount of glucagon (Boehringer Ingelheim, Ridgefield, CT) were infused during the pancreatic clamp to maintain euglycemia of approximately 5 mmol/L.

After stable euglycemia was established during the pancreatic clamp, four hour single-step hyperglycemic clamps at differing insulinemia were performed (protocol 1 and 3). At time 0 min, the insulin infusion was either maintained at 1.8 pmol/kg/min (protocol 1) or increased to 9 pmol/kg/min (protocol 3) and continued until 240 min (figure 1). End 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 or 11.1 mmol/L, were achieved using a modification of the glucose clamp technique (17). Individuals participating in protocols 2 and 4 underwent a 2 hr euglycemic or hypoglycemic clamp at an insulin infusion dose of 9 pmol/kg/min (32). Potassium chloride (20 mmol/l) was infused during hyperinsulinemic clamp studies to reduce insulin-induced hypokalemia. End of clamp blood samples were drawn at the same time of the day during all protocols.

2.4 Analytical Methods

The collection and processing of blood samples have been described elsewhere (13). Plasma glucose concentrations were measured in triplicate every 5 min using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Insulin was measured as previously described (50) with an interassay CV of 9%. Catecholamines were determined by HPLC (8) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. NEFA were measured using the WAKO kit adopted for use on a Packard Instrument (Meriden, CT). Glucagon was measured according to a modification of the method of Aguilar-Parada, et al. with an interassay coefficient of variation (CV) of 12% (2). Cortisol was assayed using the Clinical Assays Gamma Coat Radioimmunoassay (RIA) kit with an interassay CV of 6%. Growth hormone was determined by RIA (30) with a CV of 8.6%.

All vascular adhesion molecules were assayed using LINCO Research Kits (St. Charles, Missouri) with an interassay CV of 8.5% (VCAM-1), CV of 9.7% (ICAM-1), CV of 13.4%, (E-selectin), CV of 9.02% (IL-6), CV of 9.98 (TNF-α), respectively. PAI-1 and tissue plasminogen activator (TPA) were determined by TintElize® Platinum Kit with interassay CV of 3.3%. P-selectin was measured using the Meso Scale Discovery assay kit (Gaithersburg, MD) with a CV of 9.9%. All hormones, pro-inflammatory biomarkers and NEFA included in this report were measured using identical assay methodologies, equipment and personnel (32, 40).

2.5 Endothelial Function

Measurements of endothelial function were conducted at baseline and during the final 30 minutes of each glucose clamp as previously described (32, 40). In brief, endothelial measurements of the dominant brachial artery were measured using 2D Doppler ultrasound during reactive hyperemia and exogenous nitroglycerin administration. Flow mediated dilation was obtained by inflating the sphygmomanometric cuff around the proximal forearm. Brachial artery diameter measurements were taken at time points 30 seconds, 60 seconds, 90 seconds, 120 seconds and after cuff deflation. Then after a 15–20 minute rest period subjects received 0.4 mg sublingual nitroglycerin (as an exogenous nitric oxide donor). Additional scans were performed as above with vessel diameter measurements obtained at 1, 2, 3 and 4 minutes (22). Endothelial function measurements were performed by JP and NJ (32, 40). The coefficient of variation (CV) at baseline and end of clamp measurements was <1%.

2.6 Statistical Analysis

Data are expressed as mean ± SE and were analyzed using standard, parametric, one- and two-way analysis of variance and with repeated measures where appropriate (Graph Pad Software, Inc., San Diego, CA). Tukey's post hoc analysis was used to delineate statistical significance within each group. Data was also analyzed using paired and unpaired two-tailed t test. In all cases p value of <0.05 was accepted as statistically significant.

3. RESULTS

3.1 Glucose and Insulin

Plasma glucose was maintained at 5.0±0.1 mmol/L during euglycemic, 2.9±0.1 mmol/L during hypoglycemic and 11.1±0.1 mmol/L during the hyperglycemic clamp protocols (figure 1). Insulin levels were 194±23 pmol/L during euinsulinemia and similarly increased (848±49–988± 114 pmol/L) during the hyperinsulinemic hyperglycemic, euglycemic and hypoglycemic studies, respectively. Glucose infusion rates were 29±4.6, 29±5.4 and 71±6 μmol/kg/min during the euinsulinemic hyperglycemic, hyperinsulinemic euglycemic and the hyperinsulinemic hyperglycemic studies, respectively. Glucose infusion rates during hypoglycemia were 5.6 ±1.7 μmol/kg/min.

3.2 Neuroendocrine Counterregulatory Hormones

Baseline epinephrine, norepinephrine, glucagon, growth hormone and cortisol levels were similar at the start of all studies. Glucagon, growth hormone, epinephrine and cortisol

remained similar to baseline but norepinephrine increased during the pancreatic clamp studies. As expected glucagon fell during the hyperinsulinemic euglycemic clamps. Epinephrine, norepinephrine, cortisol, growth hormone and glucagon responses were higher ($p<0.0001–0.01$) during the final 30 min of hypoglycemia as compared to the other groups (table 1).

3.3 Intermediary Metabolism

Blood nonesterified fatty acid (NEFA) were similar at the start of all protocols and fell by similar, amounts during hyperglycemic and hyperinsulinemic clamps ($p<0.008$) (table 1).

3.4 Atherogenic Vascular Adhesion Molecules

Baseline values of VCAM, ICAM and E-selectin were similar at baseline during all four protocols (table 2). VCAM, ICAM and E-selectin responses increased ($p<0.03$-0.0001) during both euinsulinemia hyperglycemia and hypoglycemia. Responses of ICAM during euinsulemia/hyperglycemia was greater compared to hypoglycemia $P<0.007$ (figure 2) VCAM, ICAM and E-selectin responses fell compared to baseline during the hyperinsulinemic euglycemic and hyperinsulinemic hyperglycemic protocols ($P<0.05$-0.0001).

3.5 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 were increased similarly ($p<0.0002–0.02$) during euinsulinemic hyperglycemia or hypoglycemia compared to the other two protocols (figure 2). PAI-1 responses during hypoglycemia were greater ($p<0.04$) compared to increases during euinsulinemia hyperglycemia. PAI-1 and P-selectin levels fell from baseline ($p<0.02$-0.0002) during the hyperinsulinemic euglycemic and hyperglycemic protocols. TPA values remained similar to baseline during all four protocols.

3.6 Pro-inflammatory Markers

Baseline levels of IL-6, and TNF-α were similar at the start of all four protocols. IL-6 increased similarly from baseline during euinsulinemic hyperglycemic and hypoglycemic studies ($p<0.02$) (figure 2). Responses of IL-6 were reduced during hyperinsulinemic euglycemia and hyperglycemic protocols as compared to both euinsulinemic hyperglycemia and hypoglycemia ($p<0.03$-0.003). TNF-α responses during hypoglycemia were increased ($p<0.05$-0.01) compared to hyperinsulinemic euglycemia and hyperinsulinemic hyperglycemic protocols.

3.7 Endothelial Function

Flow mediated dilation during endogenous nitric oxide (NO) stimulation was significantly decreased during euinsulinemia/hyperglycemia and hypoglycemia as compared to other hyperinsulinemic hyperglycemic and euglycemic protocols ($p<0.02$-0.01) (figure 3).

There were similar changes among all protocols during exogenous NO mediated vasodilation following nitroglycerin administration. Baseline and end of clamp brachial

artery diameters were similar (0.43±0.02–0.44±0.02 cm) before flow mediated dilation measurements in all protocols.

4. DISCUSSION

In the present report we have compared the acute effects of euinsulinemic hyperglycemia, hyperinsulinemic hyperglycemia, hyperinsulinemic euglycemia and hyperinsulinemic hypoglycemia on pro-inflammatory, pro-atherothrombotic and in vivo arterial endothelial responses in healthy humans. Euinsulinemic hyperglycemia and hyperinsulinemic hypoglycemia produced similar increases in a wide range of pro-inflammatory (VCAM, E-Selectin, IL-6, TNF-α) and platelet activation (P-selectin) responses combined with similar reductions in NO mediated endothelial function. However, fibrinolytic balance was decreased by a greater extent during hypoglycemia (greater increase in PAI-1, equivalent changes in TPA) as compared to moderate hyperglycemia per se. Hyperinsulinemia prevented the acute effects of hyperglycemia but not hypoglycemia on stimulating pro-inflammatory, pro-atherothrombotic, pro-coagulant and disordered vascular endothelial responses.

Moderate hyperglycemia or hypoglycemia have been demonstrated to acutely induce a wide spectrum of pro-inflammatory, pro-atherothrombotic and pro-coagulant responses in healthy and diabetic individuals (4, 5, 16, 39, 40, 42). Additionally, both stresses can result in reductions in brachial artery flow mediated dilation. To date only one report has compared the effects of hypoglycemia with hyperglycemia on in vivo endothelial function and pro-inflammatory responses (9). This single report by Ceriello et al., performed in type 1 DM focused on two pro-inflammatory markers (IL-6 and ICAM-1) and two markers of oxidative stress. The authors suggested (as there was no formal statistical comparison) that in type 1 DM moderate hypoglycemia (2.9 mmol/L) and more severe hyperglycemia of 15 mmol/L appeared to produce equivalent inflammatory effects. In this present report we have extended the comparison to include the pathophysiologic effects of hyperglycemia or hypoglycemia on a broader group of pro-atherothrombotic, pro-inflammatory and pro-coagulant biomarkers in healthy humans.

We have previously reported in two separate articles that acute hypoglycemia or euinsulinemic hyperglycemia can have widespread systemic pro-inflammatory and pro-atherothrombotic effects (32, 40). In this present article we have compared and re-analyzed the magnitude of the above reported pro-inflammatory effects caused by either moderate hyperglycemia or hypoglycemia and also compared the effects of insulin per se on modulating the acute deleterious actions of differing glycemia on in vivo vascular function.

In order to evaluate the effects of hyperglycemia per se in healthy humans, an experimental design needs to be utilized that can break feedback loops between glucose and pancreatic β-cell insulin secretion. In this study we have utilized the pancreatic clamp technique with octreotide to prevent insulin (and also glucagon, growth hormone) secretion during the two hyperglycemic protocols. Hyperglycemic glucose clamp methodology was then added to the pancreatic clamp to produce stable hyperglycemia. During both protocols, basal amounts of insulin, growth hormone and glucagon were replaced to prevent any acute hormonal

deficiencies. Standard glucose clamp methodologies were used during the hyperinsulinemic euglycemic and hypoglycemic protocols. Four hours of moderate hyperglycemia per se (i.e. in the presence of basal euinsulinemia) or 2 hrs of moderate hypoglycemia produced similar increases in the atherogenic adhesion molecules VCAM-1 and E-selectin. Surprisingly, ICAM-1 responses were increased by a greater extent by hyperglycemia as compared to hypoglycemia. The mechanisms responsible for this finding will require further work. Responses of the pro-inflammatory biomarker IL-6 were also similarly increased by hyperglycemia and hypoglycemia.

P-selectin, a marker of platelet aggregation was also increased similarly during hyperglycemia and hypoglycemia. Fibrinolytic balance however was more greatly impaired by hypoglycemia as compared to hyperglycemia. PAI-1 the primary regulator of fibrinolytic balance was selectively increased by a greater extent by hypoglycemia, while TPA remained unchanged during hypoglycemia and hyperglycemia. Thus, taken together moderate hypoglycemia had greater pro-coagulant effects as compared to moderate hyperglycemia.

Insulin initially was considered to have pro-inflammatory effects (6, 19, 23, 44). Recent work from a number of laboratories, including notably, Dandona et al., have reported that acutely insulin has anti-inflammatory effects (11, 12, 16, 45). Our previous reports have also documented that insulin can have acute anti-inflammatory, pro-coagulant and pro-atherothrombotic effects (25, 32, 40). Those properties are more clearly demonstrated in the present report. All of the above biomarker responses were reduced from baseline during the hyperinsulinemic euglycemic studies and were significantly reduced as compared to hyperglycemia per se or hypoglycemia. Similarly, hyperinsulinemia was able to overcome the pro-inflammatory, pro-atherothrombotic and pro-coagulant effects of hyperglycemia leading to similar improvements in inflammatory biomarkers as compared to the hyperinsulinemic euglycemic control studies. However, although it is clear that insulin can overcome the pro-inflammatory effects of moderate hyperglycemia, it is unable to prevent the deleterious inflammatory effects of hypoglycemia. Along similar lines, there was no difference between TNF-α responses during hyperglycemia per se and hyperinulinemic euglycemia and hyperinsulinemic hyperglycemic control studies. In contrast, the effects of hypoglycemia to increase TNF-α were greater compared to the reduction occurring in the hyperinsulinemic studies. Thus, taken together when evaluating overall effects on adhesion molecules and inflammatory biomarkers it would appear that moderate hyperglycemia per se and hyperinsulinemic hypoglycemia have similar pro-inflammatory and pro-atherogenic effects.

We also investigated the effects of hyperglycemia per se, hypoglycemia and hyperinsulinemia on acute nitric oxide mediated brachial artery vasodilator mechanisms. Hyperglycemia per se and hypoglycemia produced similar inhibition of endogenously (i.e. vascular smooth muscle) NO mediated vasodilation. Exogenous NO mediated vasodilation was unaffected by hyperglycemia or hypoglycemia. Hyperinsulinemia was again able to reverse and protect against the deleterious effects of hyperglycemia per se, but not hypoglycemia on the vascular endothelium.

With regard to the potential mechanisms responsible for our findings; clearly insulin can not be implicated as a cause of the observed increased pro-inflammatory, pro-coagulant and pro-atherothrombotic responses. In fact, insulin completely reversed and thus protected the vasculature against the deleterious effects of hyperglycemia. However, importantly hyperinsulinemia could not reverse the widespread pro-inflammatory and pro-atherothrombotic effects of hypoglycemia. Therefore, there must be either direct effects of hypoglycemia (31, 47) or indirect actions consequent on the counter regulatory responses that are causing increased pro-inflammatory and pro-atherothrombotic effects (24, 26, 46).

Cathecholamines and sympathetic nervous system drive were increased during hypoglycemia and have been implicated in causing pro-inflammatory responses in a number of pathophysiologic conditions (24, 26, 46). Growth hormone, glucagon and cortisol were also increased during hypoglycemia. All are considered to have acute anti-inflammatory properties and thus would be unlikely to have contributed to the increased pro-inflammatory response during hypoglycemia (3, 14, 20, 28, 34, 49). NEFA levels were similar during all of the study protocols. Although increased NEFA levels have been reported to have pro-inflammatory properties, it is unlikely that NEFA could have contributed to our present findings (15, 48). In addition, recent reports have also identified that hypoglycemia can directly reduce endothelial function via elevations in mitochondrial superoxide levels, thereby reducing NO bioavailability (47).

In this present study we utilized the pancreatic clamp technique with basal insulin, glucagon and growth hormone replacement. The experimental design to replace growth hormone is unique and relevant as deficiency of GH has been reported to increase PAI-1 levels (33). There are reports that octreotide may have anti-inflammatory effects (35). However, there were clear differences in the effects of euinsulinemic and hyperinsulinemic hyperglycemia on pro-inflammatory, pro-atherothrombotic and endothelial function responses, which both incorporated octreotide in the pancreatic clamp.

We have compared moderate hyperglycemia (11.1 mmol/L) with moderate hypoglycemia (2.9 mmol/L). Therefore, we can not comment on whether more severe hyperglycemia or deeper hypoglycemia would have produced greater overall pro-inflammatory responses from either hyperglycemia of hypoglycemia.

In summary, moderate hyperglycemia and moderate hypoglycemia in healthy humans can acutely induce a wide range of pro-inflammatory, pro-atherothrombotic and pro-coagulant responses coupled with reductions in endogenously NO mediated vasodilation. Fibrinolytic balance appears to be more greatly reduced by moderate hypoglycemia as compared to hyperglycemia. Insulin can prevent the broad pro-inflammatory effects of hyperglycemia but not hypoglycemia.

4.1 Conclusion

In conclusion, both moderate hyperglycemia and hypoglycemia should be considered pro-atherothrombotic stimuli in healthy man. Moderate hypoglycemia’s deleterious vascular effects cannot be prevented by insulin and may have greater pro-coagulant effects as compared to moderate hyperglycemia in healthy humans.

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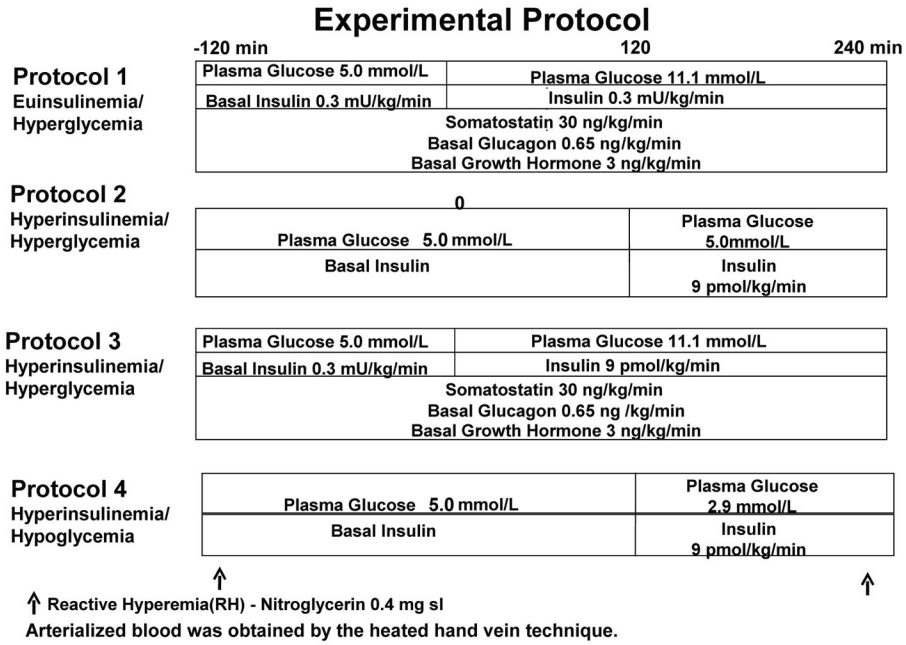

Figure 1.
Experimental protocols.

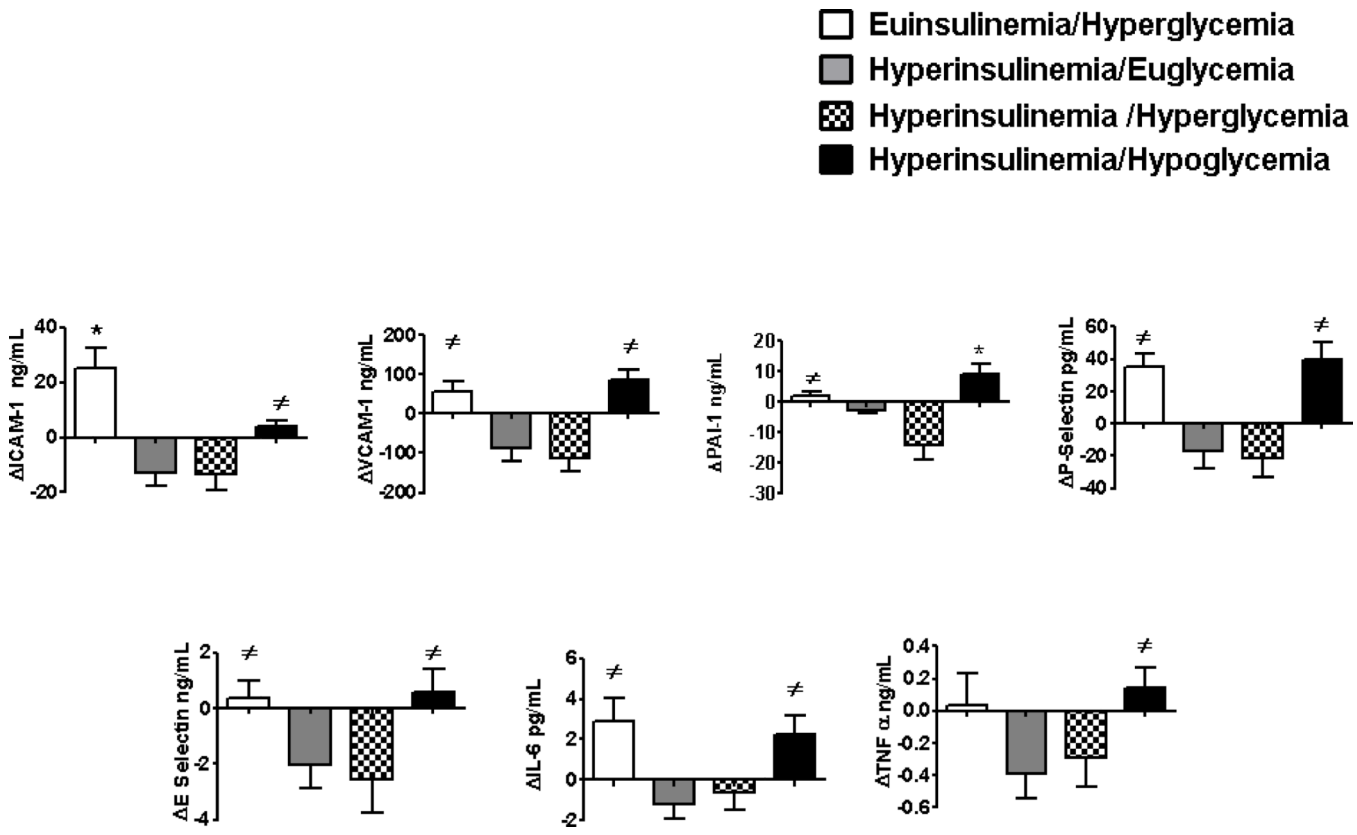

Figure 2.

Effects of euinsulinemic/hyperglycemia, hyperinsulinemic/euglycemia, hyperinsulinemic/hyperglycemia, and hyperinsulinemic/hypoglycemia on pro-inflammatory, pro-atherothrombotic, and pro-coagulant responses in overnight fasted, healthy individuals.

*p<0.04-0.0001 Significantly different from all protocols

≠p<0.05-0.0001 Significantly different from hyperinsulinemia/euglycemia and hyperinsulinemia/hyperglycemia

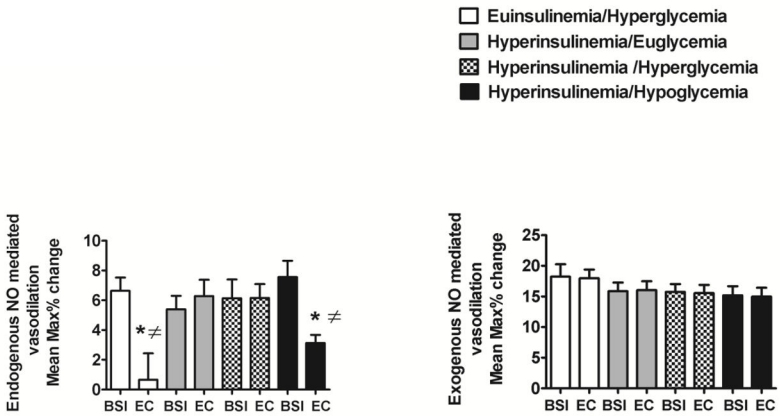

Figure 3.

Effects of euinsulinemic/hyperglycemia, hyperinsulinemic/euglycemia, hyperinsulinemic/hyperglycemia, and hyperinsulinemic/hypoglycemia on endogenous and exogenous nitric oxide (NO) mediated brachial artery vasodilation in overnight fasted, healthy individuals.

*p<0.0008-0.0001 Significantly different from baseline

≠p<0.02-0.01 Significantly different from hyperinsulinemia/euglycemia and hyperinsulinemia/hyperglycemia

Table 1

Baseline (BSL) and End of Clamp (EC) Levels of Neuroendocrine Hormones and NEFA Levels in overnight fasted healthy individuals during euinsulinemic/hyperglycemic, hyperinsulinemic/euglycemic, hyperinsulinemic/hyperglycemic and hyperinsulinemic/hypoglycemic clamps

SI units	Euins/Hypergly		Hyperins/Eugly		Hyperins/Hypergly		Hyperins/Hypo	
	BSL	EC	BSL	EC	BSL	EC	BSL	EC
GH ng/mL	2.3±0.2	2.7±0.2	2.5±0.4	2.4±0.4	2.6±0.3	2.2±0.2	3.1±0.5	28±5 *≠
Glucagon ng/L	56±6	46±5	65±5	43±3 *	65±6	49±5	57±4	164±26 *≠
Epinephrine pmol/L	114±17	168±38	190±20	189±20	140±17	156±48	184±40	3741±465 *≠
Norepinephrine pmol/L	946±111	1456±172 *	1083±80	1159±77	780±77	1178±208 *	1057±119	1966±149 *≠
Cortisol nmol/L	260±50	378±83	344±31	292±30	273±27	273±55	359±34	850±70 *≠
NEFA mmol/L	192 ± 53	88 ± 28 *	379 ± 48	103 ± 40 *	194 ± 31	65 ± 16 *	288±32	110±19 *

NEFA= non esterified fatty acids

* p<0.03 -0.0001 Significantly different from BSL

≠ p<0.03 -0.0001 Significantly different from all protocols EC

Table 2

Baseline (BSL) levels of pro- inflammatory, pro-atherothrombotic and pro-coagulation markers in overnight fasted healthy individuals during euinsulinemic/hyperglycemic, hyperinsulinemic/euglycemic, hyperinsulinemic/hyperglycemic and hyperinsulinemic/hypoglycemic clamps

SI units	Euinsulinemia/Hyperglycemia	Hyperinsulinemia/Euglycemia	Hyperinsulinemia/Hyperglycemia	Hyperinsulinemia/Hypoglycemia
VCAM-1 ng/mL	737±39	838±37	752±45	819±62
ICAM-1 ng/mL	76±10	90±7	81±9	88±3
P-Selectin pg/mL	74±7	83±9	79±14	63±13
E-Selectin ng/mL	19±2	22±2	22±3	21±2
PAI-1 ng/mL	15±3	18±3	24±6	13±3
IL-6 ng/mL	16±5	12±7	20±9	12±3
tPA ng/mL	5.8±1.2	6.1±1	5.3±1.3	5 ±1