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Acute Suppression of Circulating sCD40L during Hyperglycemia and Euglycemic-Hyperinsulinemia in Healthy Young Males

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Abstract

sCD40L is a pro-atherogenic cytokine, part of the TNF superfamily and consistently associated with obesity, diabetes, and increased cardiovascular (CV) risk. While the role of sCD40L in the onset/progression of CV complications of dysmetabolic diseases may be modulated by acute and/or chronic fluctuations of plasma insulin and glucose, very little has been done to clarify this interaction. The kinetic profile of sCD40L (and, in an exploratory manner, of several immuno-modulatory factors), were measured during hyperglycemia and euglycemic-hyperinsulinemia in a group of ten healthy young males (26.8±1.4 yrs). After an overnight fast, i.v. catheters were placed in antecubital veins of both arms for blood drawing and dextrose/insulin i.v. infusions. Procedures lasted 240 min, including baseline (t = 0–60), hyperglycemia (t = 60–150; plasma glucose ~220 mg/dL via i.v. dextrose infusion); and euglycemic-hyperinsulinemia (t = 150–240; glucose infusion continued to clamp glycemic levels between 80 and 110 mg/dL; constant insulin infusion @ 1.5 mU/kg/min).

Plasma for cytokine assays was sampled at 12 separate time-points. Plasma levels of sCD40L were significantly reduced ($p < 0.01$) during hyperglycemia and euglycemic-hyperinsulinemia, paralleling the kinetic profiles of FFA and ketone bodies. This pattern was also observed in other immuno-modulatory factors (notably cortisol and EGF), while (IL-1 α , -4, -6, -9, -10, TNF- α , Eotaxin) did not change significantly. Significant reductions of the pro-atherogenic cytokine sCD40L were observed during endogenous and exogenous hyperinsulinemia, independent of prevailing glucose concentration, in young healthy males. Our data suggest a mechanism by which correct insulin action may exert a beneficial protective role against inflammation independent of its immediate glucose-lowering effect.

Keywords

sCD40L; cytokines; insulin; healthy; young males

Introduction

An increasing amount of evidence is linking the onset and progression of cardiovascular disease (CVD), including the CVD complications of dysmetabolic states such as obesity and type 1/type 2 diabetes, to a chronically increased inflammatory status (1–5). Consequently, numerous pro- and anti-inflammatory cytokines, chemokines, growth factors and other immune-modulators (CRP, IL-1 α & β , IL-6, IL-8, MCP-1, INF- γ , members of the TNF super-family, and many others) have been proposed as markers of the onset/progression of these cardiovascular complications (6–10). Among the many molecules involved in these processes, sCD40L has received considerable attention in recent years, due to its consistent association with states of increased cardiovascular risk.

sCD40L is the soluble fraction cleaved off a larger molecule, CD40L, expressed on the surface of activated platelets (11). Both molecules bind to the CD40 receptor in a number of vascular cells, including endothelial cells, inducing the expression of adhesion molecules, inflammatory cytokines (12) and pro-coagulant factors (13;14), thereby exerting a marked pro-atherogenic effect. Elevated systemic sCD40L concentrations have been repeatedly associated with a number of atherothrombotic conditions (15) including cerebral ischemia (16) and both stable and unstable acute coronary syndromes (17–19) in both men and women (20). Elevated sCD40L has also been systematically associated with dysmetabolic conditions such as obesity (21;22), impaired glucose tolerance (23), metabolic syndrome (23;24) and both type 1 (25–29) and type 2 diabetes (29–32). Further, within diabetic populations' sCD40 L levels appear most elevated in the presence of vascular conditions such as microangiopathy (28), and carotid atherosclerosis (27).

The mechanisms by which this molecule modulates inflammation in the diabetic are still unclear, and their definition is likely to provide conceptual and practical tools for the prevention of diabetic complications (33). Indirect evidence suggests that the frequent fluctuations of plasma glucose and insulin, associated with diabetes, may have a marked impact on the acute changes in inflammatory status. Hyperglycemia, in fact, even in non-diabetic subjects induces the acute elevation of IL-6 (34–36), F2 isoprostanes (37) and reactive oxygen species oxygen species (38). Insulin, on the other hand, has been shown to exert a direct anti-inflammatory effect both in type 1 (39) and type 2 diabetes (40). While two recent studies (41;42) have reported increased platelet expression of CD40L after prolonged (24h) hyperglycemia and/or hyperinsulinemia, very little is known on the effects of acute insulin or glucose fluctuations on the soluble fraction, sCD40L.

In the present study we therefore tested the hypothesis that brief hyperglycemia and/or hyperinsulinemia may acutely and significantly affect circulating levels of sCD40L, as a possible mechanism linking this molecule to the inflammatory-mediated cardiovascular risk in dysmetabolic diseases. Ten young healthy males participated in a 4-hour clamp protocol comprised of hyperglycemia of ~ 220 mg/dL followed by hyperinsulinemic-euglycemia of (plasma glucose between 80 and 110 mg/dL), during which sCD40L, as well as glucose insulin and lipid metabolism variables were sampled repeatedly. An exploratory analysis of a series of additional inflammatory markers, which may behave in a parallel fashion with sCD40L, was also performed.

Methods

All aspects of the studies were conducted at the University of California, Irvine (UCI) Institute for Clinical Translational Science (ICTS) by specialized personnel to ensure patient safety and experimental precision. The UCI Institutional Review Board (IRB) approved all protocols, and all participants signed informed consents upon enrollment.

Subjects

Ten young healthy males participated in the study. All participants were in good health at the time of the study, had no history of chronic disease and were on no medication.

Study procedures

Subjects were admitted at the UCI ICTS at ~ 7.30 a.m. after an overnight fast. Upon admission, intravenous catheters were placed in antecubital veins of both arms for subsequent blood drawing and i.v. infusion of dextrose (20% in saline) and insulin. At ~ 8.00 a.m. the study was started ($t = 0$ min); procedures lasted 240 min and were divided into baseline ($t = 0$ –60 min); transition into hyperglycemia ($t = 60$ –90 min); stable hyperglycemia ~ 220 mg/dL ($t = 90$ –150 min); transition to euglycemic-hyperinsulinemia ($t = 150$ –180 min) and euglycemic-hyperinsulinemia ~ 80–110 mg/dL ($t = 180$ –240 min) (Figure 1).

At $t = 60$ min an i.v. infusion of 20% dextrose was started at an initial rate of 10 mg/kg/min; plasma glucose was tested every 5 min (0.4 mL blood, this continued until $t = 240$) and the dextrose infusion adjusted accordingly, with the target of reaching a stable plasma glucose concentration in the 190–210 mg/dL range by $t = 90$ min; plasma glucose was then maintained at this levels until $t = 150$. Between $t = 150$ and 180, an i.v. insulin infusion was started at a rate of 1.5 mU/kg/min, while the dextrose infusion was initially reduced to help reestablishing euglycemia by $t = 180$, and then adjusted as needed to maintain euglycemic-hyperinsulinemia until $t = 240$. In addition to the 0.4 mL blood draws that occurred every 5 min from $t = 60$ to $t = 240$, 10 mL blood draws for insulin, lipids, cytokines and oxidative marker measurements were drawn at $t = 30, 60, 90, 110, 130, 140, 150, 180, 200, 220, 230$ and 240. Immediately after blood draws, a small aliquot (0.4 mL) of blood was spun on a microcentrifuge and immediately analyzed for plasma glucose by the glucose oxidase technique on a Beckman Glucose Analyzer II (Beckman Ltd, Fullerton, CA). The remaining sample was centrifuged and the supernatant aliquotted and stored at -80°C for later analysis.

Laboratory procedures

On assay day, only the minimum necessary plasma volume of each sample was thawed. All samples for each assay were assayed simultaneously; as samples had been collected over a three-month span, this meant that while some samples were assayed within a few days or weeks after collection, other were assayed up to 4 months after collection; while this time span is unlikely to have affected study results, this procedure minimized the potentially much greater variability introduced by assaying subsets of samples at different times.

Concentrations of sCD40L and other cytokine were obtained using LINCOplex Kit (LINCO Research, St. Charles, MO, Catalog # HCYTO-60K- 30 -plex) microbeads. The Intra-assay CVs are 4.8 – 9.0%, inter-assay CVs are 3.1–18.4%, and sensitivities in serum or plasma are 0.5 – 16 pg/mL. Cytokine-antibody complexes were detected using Luminex 200 (Luminex Corp., Austin, TX) and the raw data results obtained using the MasterPlex Software (Miraibio, Alameda, CA).

For other variables of interest measurements were performed with the following techniques: **FFAs**: (plasma) NEFA-ACS-ACOD Reagent System kit (Equal Diagnostics, Exton, PA). Intra-assay CV is 1.1–2.7%, sensitivity is 0.1 mEq/L. **Glycerol**: (serum or plasma) Serum Triglyceride Kit (SIGMA, MI, TR0100). The procedure involves enzymatic hydrolysis by lipase of the triglycerides to glycerol and free fatty acids. The glycerol produced is measured by coupled enzyme reactions. The absorbance at 540 nm is directly proportional to the free glycerol concentration of the sample. **Insulin**: (plasma) ELISA with the use of the DSL-10-6000 Active Insulin System kit (Diagnostic Systems Laboratories, Webster, TX). Inter-assay CV is 1.3–2.6% intra-assay CV is 5.2–6.2%, sensitivity 0.26 uIU/ml. **β -OH-butyrate** levels in serum were measured by colorimetric determination using the β -hydroxybutyrate LiquiColor assay (StanBio Laboratory, Boerne, Texas). **Cortisol**, DSL-10-2000 Active Cortisol System kit.

Statistical Analysis

Data are presented as group means $\pm$ standard error of the mean. Comparisons among the three experimental conditions (baseline, hyperglycemia, euglycemic-hyperinsulinemia) were performed using the average of the last time points for each condition (2 time points for baseline, last three 3 points for hyperglycemia and euglycemic-hyperinsulinemia), utilizing one-way repeated measure ANOVA. Main effects and interactions effects were evaluated. When overall significance was detected, a Tukey post-hoc test was performed to identify individual differences. A p -value ≤ 0.05 was considered statistically significant. All statistical procedures were performed in collaboration with the UCI GCRC staff biostatistician.

Results

sCD40L

Ten young healthy males participated in the study (age 26.8 ± 1.4 years, range 21.6 – 35.5) (Table 1). All subjects tolerated experiment wells with no complications. Circulating concentrations of sCD40L were markedly affected by experimental procedures. The mean baseline concentration (2635 ± 484 pg/mL) was significantly reduced during both the hyperglycemic and the hyperinsulinemic-euglycemic periods (1399 ± 201 pg/mL during min 130–150, and 1405 ± 332 pg/mL, $p < 0.01$). This kinetic pattern was very homogeneous across subjects, with 10 out of 10 displaying mean significant reductions of (averaging $> 50\%$ as compared to baseline) during the experimental periods.

To eliminate the possibility that the reduction in sCD40L was simply due to a time effect, in a separate set of 5 control experiments, we measured the cytokine's profile during a similar

time course, without however inducing glucose or insulin changes; in this setting systemic concentrations of sCD40L remained unchanged compared to baseline (3552 ± 582 pg/mL) at times 150 min (3455 ± 772 pg/mL) and 240 min (3817 ± 878 pg/mL).

Indicators of glucose and lipid metabolism

By experimental time $t = 90$, the mean plasma glucose has risen from a baseline value of 95 ± 0.2 mg/dL to 197 ± 4 mg/dL, and was maintained at an average concentrations of 203 ± 6 mg/dL for the following hour. Euglycemia was then re-established over the next 30 min, so that by $t = 180$ the mean plasma glucose was 100 ± 4 mg/dL; for the remaining 60 min of the study the average glucose concentration was 91.0 ± 2.8 mg/dL. Basal plasma insulin levels were 6.8 ± 1.3 μ U/mL, and increased at the end of the hyperglycemic period ($t = 140$ – 150) to 88 ± 13 μ U/mL. Exogenous insulin infusion then began at experimental time $t = 150$, maintaining hyperinsulinemia until the end of the study (average insulin concentration at $t = 220$ – 240 105 ± 7 μ U/mL) (Figure 2).

As expected, the major indices of lipid metabolism were markedly suppressed during hyperinsulinemia. The baseline fasting FFA concentration was 316 ± 19 μ M, decreased during both hyperglycemia and euglycemic-hyperinsulinemia (166 ± 29 μ M and 123 ± 25 μ M, respectively ($p < 0.0001$). Similarly, serum β -hydroxybutyrate was 19.8 ± 1.8 μ M at baseline, decreased during hyperglycemia to 13.2 ± 1.6 μ M and remained suppressed during euglycemic-hyperinsulinemia (11.2 ± 1.0 μ M, $p < 0.0001$). Changes in glycerol were less pronounced and not statistically significant (12.6 ± 0.9 μ g/mL at baseline; 12.6 ± 1.8 μ g/mL during hyperglycemia; 10.9 ± 1.3 μ g/mL during euglycemic-hyperinsulinemia).

Other inflammatory Markers

Among other immuno-modulatory factors included in our exploratory analysis, cortisol and epidermal growth factor (EGF) displayed a kinetic pattern similar to sCD40L, with significant suppression of circulating concentrations occurring during both experimental periods as compared to baseline. For cortisol, values measured during the last 20 min of hyperglycemia (24.6 ± 3.1 μ g/dL) and the last 20 min of hyperinsulinemic-euglycemia (22.7 ± 3.6 μ g/dL) were significantly lower than baseline (36.0 ± 3.8 μ g/dL, $p < 0.001$). Similarly, the measured levels of EGF were 35.4 ± 3.2 pg/mL during hyperglycemia and 35.0 ± 5.1 pg/mL during hyperinsulinemic-euglycemia were significantly lower than baseline concentrations (94 ± 26 pg/mL, $p < 0.01$).

Values for other measured immune-modulatory factors are shown in table 2. While for most variables very little change was observed, some molecules displayed overall kinetic patterns paralleling that of sCD40L and lipid metabolites, as was for instance the case with IP-10, for which the baseline levels of 145 ± 17 pg/mL decreased during hyperglycemia to 133 ± 22 pg/mL and remained suppressed during euglycemic-hyperinsulinemia (133 ± 18 pg/mL), although not statistically significant. Conversely, in some instances moderate increases were observed, as in the case of monocyte chemoattractant protein-1 (MCP-1), in which baseline levels of 106 ± 9 pg/mL increased first non-significantly during hyperglycemia to 112 ± 9 pg/mL, and then significantly during euglycemic-hyperinsulinemia (132.2 ± 11.7 pg/mL, $p < 0.02$).

Discussion

The main finding of this study was that circulating concentrations of the pro-inflammatory, pro-atherogenic cytokine sCD40L, a member of the TNF superfamily consistently associated with elevated risk of cardiovascular disease (15–19), were markedly suppressed in ten healthy young males during three hours of hyperinsulinemia, of both endogenous and exogenous origin, independent of prevailing glycemic levels. Our observations suggest a mechanism by which physiological insulin action may exert a beneficial protective role against inflammation independent of its immediate glucose-lowering effect.

While the determination of the underlying mechanisms by which sCD40L concentrations were suppressed in our experimental conditions is beyond the scope of the present work, careful observation of the concomitant changes in metabolic variables may help direct patho-physiological speculation. Glycemic levels during the study likely had little impact on sCD40L concentrations, as the initial suppression observed during hyperglycemia was unaffected by restoring and maintaining euglycemia. Rather, the sCD40L kinetic profile appeared to closely parallel that of plasma FFAs (and therefore that of FFA-derived β -OH-butyrate), while providing a mirror image of the insulin profile. As pro-inflammatory, pro-oxidant effects have been documented with even brief elevations of circulating lipids (43–45), and anti-inflammatory properties have been clearly associated with insulin (46–48), it is not difficult to predict that a combined decrease of the former and increase of the latter would result in improved inflammatory status. Insulin, however, also directly affects FFA concentrations through its anti-lipolytic effect. Therefore, the suppressive stimulus for sCD40 L could have derived from lower systemic FFA (and therefore indirectly from insulin), from a direct, FFA-independent insulin effect, from a combination of the two, or from an altogether different mechanism. It should be noted that by experimental designed hyperinsulinemia derived from endogenous insulin secretion in the first half of the study, and from exogenous insulin infusion in the latter half, suggesting that not the insulin secretion process, but simply insulin levels and its downstream direct or indirect effects are likely responsible for the reported observations. Additional work is obviously required to shed more light on these issues.

To our knowledge, the only prior study investigating the effect of glucose and insulin fluctuations on the CD40L molecule is that reported in two papers by Vaidyula et al (41;42), in which, in apparent contrast with our findings, an increase in platelet CD40L expression was detected after 24 h of continuous hyperglycemia and hyperinsulinemia, generated independently or in combination. It should be noted, however, that the experimental conditions in this study are extremely different from ours. First, the experimental intervention was very prolonged (24 hours), and while the levels of hyperglycemia are comparable to those from our study, hyperinsulinemia was over 2-fold higher (with mean, supra-physiological levels of $\sim 200 \mu\text{U/ml}$). As only differences between pre- and end-study were reported, this means that the observed increase in CD40L platelet expression occurred after a very long exposure to extremely accelerated glucose turnover, posing a disproportionately greater strain on the organism's metabolic homeostasis than in our experiment. Conceptually, earlier in the study transient reductions in CD40L, similar to those observed by our group, may have occurred, and gradually transitioned to alterations in

the opposite direction, after the adaptability of the system was saturated. A bi-phasic inflammatory response to stress is not unusual, as recently shown in our laboratory during several studies utilizing intense exercise as a pro-inflammatory stimulus; the large majority of 18 tested inflammatory mediators displayed a late exercise-induced increase that was preceded by a transient below-baseline drop (49;50). Further, it should be noted that only platelet CD40L expressions were reported, not circulating sCD40L concentrations. The correlation between the two during such a prolonged metabolic manipulation (indeed during any type of metabolic manipulation) is largely unexplored at the present time. In previous studies, however, discrepancies between circulating sCD40L concentrations and CD40/CD40L surface expression have been reported; plasma sCD40L, for instance, was significantly elevated in a group of type 1 diabetic patients despite CD40/CD40L surface expression in platelets and T lymphocytes similar to controls (31). It is therefore possible that Vaidyula's studies and ours are just displaying two distinct and not necessarily incompatible aspects of the same, complex adaptive mechanism. Vaidyula's experimental conditions may be considered to reflect the chronic metabolic state of a patient with type 2 diabetes of relatively recent diagnosis. Prolonged hyperglycemia is present, and so is prolonged hyperinsulinemia, reflecting a pancreas overburdened by peripheral insulin resistance but still able to attempt compensatory insulin hyper-secretion; this metabolic state has been clearly associated with increased inflammatory markers, including sCD40L. Our experimental conditions, on the other hand, appears to reflect a healthy response to transient fluctuations of carbohydrate metabolism. The physiological, reactive hyperinsulinemia corrects the hyperglycemic episode, and both the direct anti-inflammatory effect of moderately increased insulin concentration, and the indirect effect of suppressed lipid and ketone body concentrations collude to temporarily improve systemic inflammatory status.

While the main focus of the study was to detect changes in sCD40L, it is well known that pro- and anti-inflammatory changes often comprise the coordinated activation of several, indeed often numerous cytokines, chemokines and other immune-modulatory factors. To gain a preliminary idea of which other molecules may be implicated in these processes, therefore, we ran an exploratory analysis of a number of other potentially interacting variables. Interestingly, while most tested molecules were only modestly, and mostly non-significantly affected (Table 2), epidermal growth factor (EGF) displayed a kinetic behavior which, similar to sCD40L, was the mirror image of the insulin profile. EGF is known to be potentially involved in multiple aspect of diabetes, being elevated in the saliva of type 1 diabetics (51), and possibly modulating diabetic wound healing (52) and regulating pancreatic β -cell growth and regeneration (53). Because of its effects in the pancreas, in particular, EGF has been shown to exert an insulin- like effect in diabetic NOD mice, reverting hyperglycemia and increasing β -cell mass (54). It does not appear unreasonable, therefore, that EGF concentrations may be reduced by hyperinsulinemia; to our knowledge, however, regulation of this molecule in such an acute setting has never before been reported.

Caution should be always used in interpreting results from complex assays such as that for sCD40L, as analytical issues may significantly alter results. In a recent paper by Ivandic et al, for instance (55), it was pointed out how changes in sCD40L concentrations may derive from in vitro platelet activation during sample preparation, masking changes actually occurring in vivo if pre-analytical conditions are not standardized. These considerations,

however, are unlikely to apply to our results, as our main findings consist of changes across multiple samples within each subject, which were all collected, stored and analyzed by the same personnel, in identical conditions and with identical procedures.

In conclusion, exposure of ten healthy young males to sustained hyperinsulinemia of ~ 100 μ U/mL, both spontaneous and of exogenous origin, resulted in a ~ 50%, significant suppression of circulating levels of the pro-atherogenic factor sCD40L, independent of prevailing glycemia. Modulation of sCD40L may therefore be one of the mechanisms by which insulin exerts its anti-inflammatory effects, supporting the concept that this molecule should be a major target for preventive and therapeutic interventions in insulin resistant states such as obesity, glucose intolerance, metabolic syndrome and diabetes.

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Figure 1.
Schematic of experimental design.

Figure 2.

Kinetic profiles of plasma glucose, insulin, FFAs and sCD40L in 10 healthy young males at baseline and during hyperglycemia of ~ 220 mg/dL or hyperinsulinemic euglycemia. Data are group means $\pm$ SEM.

Data are group means $\pm$ SE in 10 healthy males. Plasma glucose changes were induced via i.v. infusion; increases in insulin concentrations resulted in opposite changes in lipid variables (FFA), paralleled by similar decreased profiles of pro-atherogenic molecules sCD40L.

Figure 3.

Plasma sCD40L, EGF and cortisol in 10 healthy young males at baseline, and during the last 20 of 90 min of hyperglycemia of ~ 220 mg/dL, and the last 20 of 90 min of hyperinsulinemic euglycemia. Data are group means $\pm$ SEM.

Data are group means $\pm$ SE. White bars are baseline, black bars are hyperglycemia, gray bars are euglycemic-hyperinsulinemia. † signify $p < 0.05$ euglycemic-hyperinsulinemia vs. baseline, ‡ signify $p < 0.05$ euglycemic-hyperinsulinemia vs. hyperglycemia.

Figure 4.

Plasma FFAs, glycerol and β -OH-butyrate in 10 healthy young males at baseline, and during the last 20 of 90 min of hyperglycemia of ~ 220 mg/dL, and the last 20 of 90 min of hyperinsulinemic euglycemia. Data are group means $\pm$ SEM.

Data are group means $\pm$ SE in 20 healthy adults. Plasma glucose changes were induced via i.v. infusion; increases in insulin concentrations resulted in opposite changes in lipid variables (FFA), paralleled by similar decreased profiles of pro-atherogenic molecules (sCD40L, EGF).

Table 1

Demographic characteristics

Age (Yr)	Height (cm)	Weight (kg)	BMI (kg/m ²)
26.8 ± 1.4	176.3 ± 2.4	79.4 ± 3.0	25.6 ± 1.0

Data are means ± SE.

TABLE 2

Absolute concentrations of inflammatory mediators, 20-min average

Cytokine	Baseline	Hyperglycemia	Euglycemic-Hyperinsulinemia	p-values
IL-1a	855.3 ± 310.0	833.3 ± 264.7	745.5 ± 234.7	
IL-1ra	89.2 ± 16.0	117.8 ± 21.2	106.2 ± 29.7	
IL-2	10.4 ± 2.8	13.7 ± 5.2	13.5 ± 4.9	
IL-4	869.2 ± 376.3	892.8 ± 348.7	729.6 ± 283.7	
IL-5	22.9 ± 8.0	24.7 ± 8.9	18.9 ± 6.9	
IL-6	193.3 ± 50.8	195.5 ± 52.0	175.5 ± 47.1	
IL-8	40.8 ± 12.8	38.4 ± 13.1	31.2 ± 10.9	
IL-10	51.4 ± 10.7	47.8 ± 14.6	46.0 ± 12.1	
IL-12p40	286.1 ± 39.9	298.8 ± 46.6	302.6 ± 40.8	
IL-12p70	11.7 ± 2.1	12.9 ± 2.6	12.5 ± 2.8	
IL-13	12.8 ± 2.6	13.7 ± 2.9	11.6 ± 1.9	
IL-17	14.7 ± 3.8	15.6 ± 4.7	15.8 ± 4.6	
IFN-γ	15.8 ± 2.8	16.8 ± 3.2	20.3 ± 5.3	
GM-CSF	8.8 ± 3.1	8.0 ± 2.7	8.2 ± 2.7	
Exotaxin	78.5 ± 21.5	78.9 ± 23.8	68.4 ± 22.2	
EGF	94.3 ± 25.6	35.4 ± 3.2*	35.0 ± 5.1 [†]	* [†] <0.009
sCD40L	2635.4 ± 483.5	1398.8 ± 200.9*	1405.4 ± 331.7 [†]	* [†] <0.005
MCP-1	106.2 ± 8.9	112.4 ± 8.5	132.2 ± 11.7 [†]	[†] <0.017
MIP-1a	16.0 ± 2.1	18.0 ± 3.2	19 ± 3.4	
MIP-1β	71.8 ± 10.3	68.5 ± 12.3	79.8 ± 14.3	
IP-10	145.2 ± 16.8	132.8 ± 21.5	133.0 ± 17.6	

Data are means ± SE. Units are pg/mL.

* Hyperglycemia vs Baseline,

[†] Euglycemic-Hyperinsulinemia vs Baseline