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Acute hyperinsulinemia raises plasma interleukin-6 in both nondiabetic and type 2 diabetes mellitus subjects, and this effect is inversely associated with body mass index

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Abstract

Hyperinsulinemia is a characteristic of type 2 diabetes mellitus (T2DM) and is believed to play a role in the low-grade inflammation seen in T2DM. The main aim was to study the effect of hyperinsulinemia on adipokines in individuals with different levels of insulin resistance, glycemia, and obesity. Three groups of sex-matched subjects were studied: young healthy subjects (YS; n = 10; mean age, 26 years; body mass index [BMI], 22 kg/m^2), patients with T2DM (DS; n = 10; 61 years; BMI, 27 kg/m^2), and age- and BMI-matched controls to DS (CS; n = 10; 60 years; BMI, 27 kg/m^2). Plasma concentrations of adipokines were measured during a hyperinsulinemic euglycemic clamp lasting 4 hours. Moreover, insulin-stimulated glucose uptake in isolated adipocytes was analyzed to address adipose tissue insulin sensitivity. Plasma interleukin (IL)-6 increased significantly ($P \leq .01$) in all 3 groups during hyperinsulinemia. However, the increase was smaller in both DS ($P = .06$) and CS ($P < .05$) compared with YS (~2.5-fold vs ~4-fold). A significant increase of plasma tumor necrosis factor (TNF) α was observed only in YS. There were only minor or inconsistent effects on adiponectin, leptin, and high-sensitivity C-reactive protein levels during hyperinsulinemia. Insulin-induced rise in IL-6 correlated negatively to BMI ($P = .001$), waist to hip ratio ($P = .05$), and baseline (fasting) insulin ($P = .03$) and IL-6 ($P = .02$) levels and positively to insulin-stimulated glucose uptake in isolated adipocytes ($P = .07$). There was no association with age or insulin sensitivity. In a multivariate analysis, also including T2DM/no T2DM, an independent correlation (inverse) was found only between BMI and fold change of IL-6 ($r^2 = 0.41$ for model, $P < .005$). Hyperinsulinemia per se can produce an increase in plasma IL-6 and TNFα, and this can potentially contribute to the low-grade inflammation seen in obesity and T2DM. However, obesity seems to attenuate the ability of an acute increase in insulin to further raise circulating levels of IL-6 and possibly TNFα.

1. Introduction

It is recognized that obesity and type 2 diabetes mellitus (T2DM) are associated with chronic low-grade inflammation, and adipose tissue appears to represent an important site of inflammation that is also linked to dysregulated release of adipokines [1-4].

Interleukin (IL)-6 is released from macrophages present in adipose tissue as well as other sites and also from adipocytes and skeletal muscle [1,5,6]. In vitro and in vivo work has shown that IL-6 gene expression may be regulated by insulin [7]. Hyperinsulinemia is reported to produce a significant increase in adipose tissue IL-6 messenger RNA (mRNA) and in circulating levels of IL-6, but not in skeletal muscle IL-6 mRNA [8-10]. In addition, circulating levels of IL-6 correlate well with central obesity [11]. Tumor necrosis

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factor (TNF) α levels are associated with insulin resistance, and this cytokine promotes serine phosphorylation of insulin receptor substrate 1 (IRS-1) that impairs insulin signaling [12,13]. Similar to IL-6, TNFα mRNA in adipose tissue was reported to increase during hyperinsulinemia [10]. In contrast, no change was observed in circulating levels; and TNFα mRNA in muscle decreased during hyperinsulinemia.

Insulin peaks after meals may be important in regulating leptin production [14,15]. The acute effect of insulin on circulating leptin levels in humans is, however, not clear, as there are reports indicating a stimulating effect as well as no effect [16-18].

Adiponectin is negatively correlated with insulin sensitivity and fasting plasma insulin concentrations, and adiponectin appears to enhance insulin action. In contrast to leptin, adiponectin levels in plasma vary little between feeding and fasting. The mechanism by which adiponectin can modulate insulin action is largely unknown [14]. Conversely, during euglycemic hyperinsulinemic clamps, a 10% to 15% fall in circulating adiponectin levels has been reported [16,19].

Although regulation of adipokines by insulin has been studied before, the interaction is not yet well understood; and only a few studies are available. There are scarce data on the effect of hyperinsulinemia on circulating concentrations of adipokines in healthy humans, and there are essentially no such results in patients with T2DM. We therefore addressed this question by studying the effect of prolonged experimental hyperinsulinemia (4 hours) on levels of adipokines in blood. To our knowledge, this is the first study addressing the effect on adipokines of a prolonged clamp at high insulin concentrations in both T2DM subjects and healthy controls.

2. Subjects and methods

2.1. Subject groups

The study was approved by the Ethics Committee of Umeå University. The subjects reported here were studied as part of a larger study in which subjects underwent euglycemic hyperinsulinemic clamps on a number of occasions (T Ruge t al, to be published) to study effects on lipoprotein lipase activity.

Subjects consisted of volunteers who all gave informed consent. Three groups (matched with respect to sex; 4 men and 6 women in each group) were recruited for this study: (1) young healthy subjects (YS), (2) T2DM patients (DS), and (3) controls matched for age and BMI to the diabetic patient group (CS). All subjects were nonsmokers, and none of the subjects had signs of alcohol overconsumption. Subjects were asked to refrain from vigorous exercise and from alcohol intake for 48 hours before the investigation. Most of the young subjects were students at Umeå University, and none of them had any chronic disease or were treated with any regular medication. The diabetic subjects were all treated with oral antidiabetic medication (metformin or repaglinide), and their glucose control had been stable over the previous 6 months (hemoglobin A_{1c} [HbA_{1c}], 6.6% ± 0.2%; Swedish standard, reference range, 3.6%-5.3%). No difference in blood pressure between CS and DS was observed (128 ± 14/79 ± 9 and 132 ± 20/72 ± 9 mm Hg for CS and DS, respectively). The T2DM duration ranged from 3 to 13 years. None of the subjects with T2DM had any clinically apparent complications due to their T2DM. One of the DS was taking low-dose diuretic therapy for hypertension, and 1 female subject was taking hormone replacement therapy. Three female subjects in the CS group were receiving hormone replacement therapy; but otherwise, all CS were healthy and were not taking any medications, except for 1 subject who was treated with a low-dose diuretic for hypertension.

Body composition (amount of lean and fat body mass) was estimated using the bioelectrical impedance method (Bodystat 1500; Bodystat, Douglas, Isle of Man).

2.2. Protocol

The studies were performed at the Metabolic Unit of the Umeå University Hospital. Subjects attending the studies were all fasted overnight for 10 hours. All studies started at 8:00 AM, and subjects refrained from any intake of medication on the morning of the study day.

In vivo insulin sensitivity was assessed with the euglycemic hyperinsulinemic clamp technique. During the studies, subjects were resting in a comfortable bed in a room with the temperature maintained between 24°C and 26°C. Two polytetrafluoroethylene cannulae (Venflon; Viggo, Helsinborg, Sweden) were positioned intravenously, one in an antecubital vein for all infusions and the other one inserted distally in a vein in the contralateral arm from which arterialized blood samples were taken. The forearm that was used to draw blood samples was heated with electric pads to arterialize venous blood. Semisynthetic insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused as a priming dose for the first 10 minutes, followed by a constant infusion at 56 mU m^{-2} min^{-1} ending after a total of 240 minutes. In parallel, a 20% glucose infusion was started; and the infusion rate was adjusted to maintain a steady-state blood glucose level of 5.0 mmol/L. The blood glucose concentration was determined at 5-minute intervals. Blood samples for determination of serum insulin were obtained at the start and then at 60, 90, 120, 180, and 240 minutes of the clamp. Serum and plasma samples were centrifuged within 30 minutes and stored at −80°C until analyzed. The rate of glucose infusion served as a measure of whole-body insulin sensitivity; and during the conditions used, it will mainly represent glucose uptake in tissues, primarily skeletal muscle. The M-value (milligram per kilogram of lean body mass [LBM] per minute) was calculated by dividing the amount of glucose infused by lean body weight and time. The period used for these calculations was between 100 and 160 minutes, when steady state was achieved.

2.3. Analytical procedures

Blood glucose concentrations were determined by the HemoCue glucose system (HemoCue, Ängelholm, Sweden), HbA_{1c} was measured by high-pressure liquid chromatography (Integral 4000; Bio-Rad, Anaheim, CA), and values were calibrated according to the national Swedish standard (MonoS; reference range, 3.6-5.3). High-sensitivity C-reactive protein (hs-CRP) was assessed by CRP HS Tinaquant kit (Roche Diagnostics, Indianapolis, IN), insulin was assessed by a microparticle enzyme immunoassay (Abbott Imx; Abbott Laboratories, Abbott Park, IL), and C-peptide was analyzed with the routine method of the Laboratory of Clinical Chemistry at the Umeå University Hospital. Plasma TNFα and IL-6 levels were determined using high-sensitivity commercial immunoassay kits (Quantikine HS Human TNFα Immunoassay and Quantikine HS Human IL-6 Immunoassay, respectively; R&D System, Minneapolis, MN). Plasma adiponectin and leptin levels were determined using a commercial human adiponectin and leptin enzyme-linked immunosorbent assay kit of sandwich type (Linco Research, St Charles, MO).

2.4. Adipose tissue biopsies

After dermal local anesthesia, a needle biopsy (2-3 g) was taken from the subcutaneous adipose tissue of the lower abdomen. This was performed just before and after 240 minutes of the hyperinsulinemic clamp. A part of each biopsy was used for acute experiments to study uptake of glucose in isolated adipocytes. Prewarmed medium was used to wash the fat tissue, and blood clots were taken away. Isolated fat cells were obtained by shaking the tissue in polypropylene containers at 37°C for 1 hour in medium 199 containing 5.6 mmol/L glucose, 40 mg/mL bovine serum albumin (BSA), and 0.6 mg/mL collagenase. The cells were then filtered through a nylon mesh and washed 4 times with fresh medium and then used for experiments. Adipocyte cell size and number were measured as previously described [20]. Glucose uptake was performed as previously described [21]. Cells were incubated without glucose at 37°C in vials containing medium 199, BSA (4%), adenosine deaminase (1 U/mL), N^6-(*R*-phenyl-isopropyl)adenosine (1 μmol/mL), and insulin (0-1000 μU/mL). After 15 minutes, D-(*U*-^{14}C) glucose (0.7-1.0 μmol/L) was added. After another 60 minutes, cells and medium were transferred to prechilled tubes; and the reaction was terminated as the cells were separated from the glucose-containing medium by centrifugation through silicone oil. Cell-associated radioactivity was determined by scintillation counting. Cellular glucose uptake was expressed as glucose clearance and was calculated with the following formula: [cell-associated radioactivity × volume]/[radioactivity of medium × cell number × time]. Under these experimental conditions, glucose uptake is mainly determined by the rate of transmembrane glucose transport. D-(*U*-^{14}C)glucose (specific activity, 200-300 mCi/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, United Kingdom). Bovine serum albumin (fraction V) and N^6-(*R*-phenyl-isopropyl)adenosine were obtained from Sigma-Aldrich (Stockholm, Sweden). Adenosine deaminase and collagenase A were from Roche Diagnostics (Bromma, Sweden). Hanks medium 199 was obtained from Invitrogen, Life Technologies (Groningen, the Netherlands); and human insulin (Actrapid, 100 U/mL) was obtained from Novo Nordisk.

2.5. RNA extraction and complementary DNA synthesis

The biopsies were washed free of blood, weighed, diluted with buffer A, and frozen at −70°C for subsequent homogenization. Buffer A, used for homogenization of the biopsies, contained 0.025 mol/L NH_3, 5 mmol/L Na_2EDTA, and per milliliter: 1 mg BSA, 10 mg Triton X-100, 1 mg sodium dodecyl sulfate, and 5 IU heparin. For inhibition of proteases, 1 Complete Mini tab (Roche) was added to 50 mL of this buffer; and the pH was adjusted to 8.2. Adipose tissue was homogenized as described previously [22] and stored at −80°C. Two hundred nanograms of total RNA, extracted using TRIzol (Invitrogen Life Technologies, Paisley, UK) and isolated in accordance with the manufacturer's instructions and treated with DNA-free kit (Ambion, Austin, TX), was reverse transcribed using the SuperScript First-Strand Synthesis System (Invitrogen Life Technologies) and stored at −20°C until further use. For IL-6 and TNFα mRNA determination, 2 μL of first-strand complementary DNA was used in a final volume of 25 μL; complementary DNA was diluted 4 times for endogenous control determination, with optimal concentrations of primers and probes [23]. Polymerase chain reaction was carried out in triplicate for all samples. Primers and probes were from Invitrogen and TAG (Copenhagen, Denmark), respectively. The $2^{-\Delta\Delta Ct}$ method (User Bulletin no. 2, ABI Prism 7700; Applied Biosystems, Foster City, CA) was used for relative quantitation. Values were expressed as fold changes in the target gene normalized to the reference gene (β-actin) and related to the expression of the untreated control. Polymerase chain reaction was carried out in triplicate for all samples.

2.6. Statistical analyses

Correlations were determined with Spearman correlation coefficient, a rank-based measure. Analyses of mean differences were initially performed with standard linear regression. Variables not normally distributed (adipokines and hs-CRP) were logarithmically transformed before analyses. Correlation of within-subject longitudinal measurements during the euglycemic clamp was addressed with the use of generalized estimating equations [24]. Tests of parameters within these models were performed with generalized Wald tests [25]. This yields test statistics that are simply dependent data analogues of analysis of covariance test statistics. In the multivariate model, baseline variables that were each correlated to IL-6 fold changes in the univariate analysis ($P < .1$) were then included as

Table 1
Anthropometric and metabolic characteristics of subjects

	YS (n = 10)	CS (n = 10)	DS (n = 10)
Age (y)	26 ± 2	60 ± 2†	61 ± 3§
BMI (kg/m^2)	22.5 ± 1.0	27.4 ± 1.0†	27.5 ± 1.1§
WHR	0.82 ± 0.02	0.88 ± 0.02*	0.92 ± 0.02§
Fat mass (%)	20 ± 2	36 ± 3†	36 ± 2§
LBM (%)	80 ± 2	64 ± 3†	64 ± 2§
Fasting serum glucose (mmol/L)	4.1 ± 0.1	4.5 ± 0.2†	8.0 ± 0.4‡,§
Blood HbA_{1c} (%)	NC	4.3 ± 0.1	6.6 ± 0.2‡
Fasting serum insulin (mU/L)	5.0 ± 0.6	7.0 ± 0.7†	10.0 ± 0.1‡,§
Insulin level during clamp (mU/L)	95 ± 10	102 ± 10	121 ± 30
M-value (mg kg^{-1} LBM min^{-1})	12.0 ± 0.6	12.3 ± 0.5	5.8 ± 0.4‡,§

Data are expressed as means ± SEM. n = 10 for all groups. Student unpaired *t* test was used for analyses of differences between groups. YS indicates young subjects; CS, control subjects to DS; DS, subjects with type 2 diabetes; NC, not collected.

* $P < .05$ when CS were compared with YS.
† $P < .01$ when CS were compared with YS.
‡ $P < .01$ when DS were compared with CS.
§ $P < .01$ when DS were compared with YS.

potential predictors; and Mallows C-p statistic [26] was used to select the best model. *P* values less than .05 were considered statistically significant.

3. Results

3.1. Metabolic and anthropometric characteristics

Metabolic characteristics of the participants are summarized in Table 1. As expected, DS had impaired whole-body glucose turnover compared with both CS and YS. Diabetic subjects and CS had significantly higher amounts of body fat, increased waist to hip ratio (WHR), and lower amounts of LBM compared with YS.

Steady-state blood glucose levels during the clamps were around 5.0 (range, 4.8-5.2) mmol/L for all subjects (data not shown). During the hyperinsulinemic euglycemic clamp, plasma insulin concentrations tended to be higher in DS compared with CS and YS (not significant); and this may reflect an impaired insulin clearance in DS. Glucose infusion rates calculated per kilogram of LBM (M-values) obtained at steady state during the clamps were significantly lower for DS compared with CS and YS.

3.2. Circulating concentrations of adipokines before and during the euglycemic clamp

These data are summarized in Fig. 1 and Table 2. Baseline IL-6 levels were higher in DS ($P = .01$) and, nonsignificantly, in CS ($P = .07$) compared with YS. By the end of the hyperinsulinemic euglycemic clamp, that is, at 240 minutes, plasma concentrations of IL-6 increased by about 2.5-fold in CS and DS ($P < .01$) and by around 4-fold in YS ($P = .01$). Thus, YS displayed a higher relative IL-6 increase than DS ($P = .06$) and CS ($P < .05$). The concentrations at the intermediate time point of 120 minutes were not statistically different from baseline. At the end of the clamp, plasma IL-6 concentrations were similar in all 3 groups.

Baseline concentrations of TNFα were higher, albeit not significantly, in DS and CS compared with YS (Table 2). At the end of the clamp, a significant increase was seen only in YS ($P = .02$); and the final level did not differ from that of CS and DS. Although hs-CRP levels experienced no significant changes during the clamp within groups, there were significant baseline differences between the groups (Table 2). Diabetic subjects had the highest baseline values, followed by CS ($P = .05$) and YS ($P = .02$). These group differences still remained by the end of the clamp. Leptin concentrations were highest in DS, but only CS experienced a significant rise in leptin levels during the clamp (about 20%, $P = .02$). Young healthy subjects had lower

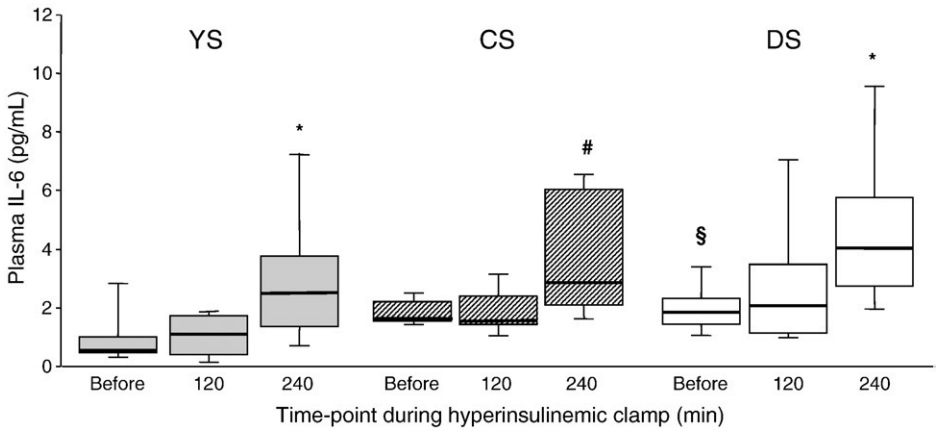

Fig. 1. Box plot showing the increase of IL-6 levels during hyperinsulinemia compared with baseline. The horizontal lines represent the median and the 10th, 25th, 75th, and 90th percentiles, respectively. YS indicates young subjects; CS, control subjects to DS; DS, subjects with type 2 diabetes. *$P < .01$, #$P = .01$ for relative increase vs baseline value. §$P < .05$ for DS baseline values compared with YS baseline values.

Table 2
Levels of adipokines and hs-CRP before and at 240 minutes of hyperinsulinemia.

	YS		CS		DS	
	0 min	240 min	0 min	240 min	0 min	240 min
IL-6 (pg/mL)	0.66	2.60†	1.34	2.72*	1.86‡	4.05†
	0.57-1.09	1.74-3.82	1.23-1.88	2.03-5.50	1.49-2.18	3.01-4.80
Adiponectin (μg/mL)	10.0	9.3	14.1	13.0*	9.3§	9.4§
	6.3-11.3	9.2-9.8	11.4-21.0	9.7-16.8	6.3-12.6	5.2-10.8
Leptin (ng/mL)	5.2	6.7	12.6	14.9*,‖	17.0‡	17.9‡
	4.7-7.3	5.5-7.5	8.3-18.5	8.3-21.7	9.3-20.1	9.9-21.9
TNFα (pg/mL)	0.71	2.33*	2.39	2.59	2.18	2.88
	0.34-1.29	1.80-3.49	1.40-4.04	2.02-3.65	1.30-4.33	2.27-4.96
hs-CRP (mg/L)	0.47	0.55	1.65‖	1.65 ‖	2.95§,‡	3.05§,‡
	0.32-0.84	0.34-0.91	0.87-2.13	0.81-2.13	2.13-4.40	2.18-4.83

Data are expressed as median and interquartile range. n = 10 for all measures, except for adiponectin, leptin, and hs-CRP in YS, n = 8. YS indicates young subjects; CS, control subjects to DS; DS, subjects with type 2 diabetes.

* $P < .05$ compared with baseline.

† $P < ..01$ compared with baseline.

‡ $P < .05$ when DS were compared with YS.

§ $P < .05$ when DS were compared with CS.

‖ $P < .05$ when CS were compared with YS.

values than the other 2 groups at both baseline and after 240 minutes ($P = .02$ when compared with DS and $P = .05$ when compared with CS). No significant difference was observed between CS and DS. Simultaneously, CS had a significant drop in adiponectin during the clamp (by 7%, $P = .03$). Their baseline values were significantly higher than DS but not YS, a pattern that was still true after 240 minutes of hyperinsulinemia. None of the measured adipokines and inflammatory mediators displayed any significant change at 120 minutes of hyperinsulinemia compared with baseline (data not shown and Fig. 1).

3.3. Correlations between adipokine levels and other variables

Linear regression analyses showed positive correlations of baseline concentrations of IL-6 and leptin with BMI ($P < .05$, $r^2 = 0.20$ and $P < .05$, $r^2 = 0.17$, respectively) and body fat ($P < .01$, $r^2 = 0.24$ and $P < .01$, $r^2 = 0.44$, respectively) when data from all subjects were analyzed together. However, no significant correlations between TNFα and BMI or body fat could be demonstrated.

Considering all subjects together, the relative change in IL-6 during hyperinsulinemic clamp was negatively correlated with BMI, WHR, baseline IL-6, and fasting insulin (Table 3). Sex and age had no significant impact on the fold change in circulating IL-6. A trend toward a positive correlation between insulin-stimulated glucose uptake in isolated adipocytes (see below) and fold change in IL-6 was observed. To examine the impact of these anthropometric and metabolic parameters on fold-change in IL-6, a multivariate model also including sex, age, and recognized T2DM or not was constructed. Here we found that only BMI was independently associated (inversely) with fold change of IL-6 ($r^2 = 0.41$ for model, $P < .005$).

We could not find any significant correlations between fold change of TNFα, adiponectin, leptin, or hs-CRP during hyperinsulinemia and any of the other analyzed anthropometric or biochemical variables either when all subjects were taken together or when the groups were analyzed separately.

3.4. In vitro analyses of adipose tissue

Fat cells were significantly ($P < .001$) smaller in YS compared with CS and DS (mean diameter ± SEM, 89.1 ± 2.5 μm in YS, 107.6 ± 4.1 μm in CS, and 106.9 ± 1.8 μm in DS). Insulin-stimulated (1000 mU/L) glucose uptake (expressed as percentage of basal nonstimulated glucose uptake) in isolated subcutaneous adipocytes was lowest in DS (175% ± 10%), highest in YS (353% ± 61%), and intermediate in CS (212% ± 14%) ($P = .01$ for DS compared with YS and $P = .05$ for CS vs YS and for CS vs DS).

Measurements of adipose tissue mRNA levels for IL-6 and TNFα were performed only in a subset of subjects (n = 5

Table 3
Results from univariate analyses on all subjects with fold increase of IL-6 during hyperinsulinemia as dependent variable

	r	P value
Age	−0.08	.68
WHR	−0.37	.05
BMI	−0.57	.001
Body fat %	−0.11	.59
Baseline IL-6	−0.45	.02
Fasting insulin	−0.42	.03
Fasting glucose	−0.25	.23
M-value	0.15	.42
AMGU	0.35	.07
Fat cell size	−0.30	.11

AMGU indicates maximal insulin-stimulated glucose uptake in adipocytes (percentage of basal).

for YS, n = 4 for CS, and n = 4 for DS), and this was due to insufficient amount of tissue in several samples. There were no significant changes in IL-6 mRNA expression in adipose tissue from any of the groups during the clamp (data not shown); and for all available subjects taken together, the median fold change relative to baseline was 0.65 (interquartile range, 0.33-1.73). There was an increase in TNFα mRNA expression in YS during hyperinsulinemia (P = .009), but not in any other group. The fold changes (relative to baseline value) in YS, CS, and DS were 2.8 (1.8-7.9), 1.2 (0.9-1.4), and 0.9 (0.5-1.8), respectively.

4. Discussion

In this study, we have addressed the acute effects of insulin on circulating adipokines. Our main finding was that acute hyperinsulinemia for 240 minutes caused a rise in IL-6 levels in all groups studied. No such effect was observed at 120 minutes of hyperinsulinemia. In univariate analyses, the increase of IL-6 during the clamp was inversely associated with obesity and with measures of insulin resistance.

Our data support that acute insulin infusion has a very limited, if any, enhancing effect on circulating leptin levels [16-18]. With respect to adiponectin, 2 previous reports suggests a small decrease of circulating levels during acute insulin infusion [16,19]. The magnitude of reduction in our study is in agreement with these reports, that is, around 10%. However, we only observed this effect in CS. Taken together, the present and previous results suggest that insulin has no significant acute effects on circulating leptin and only limited effects on adiponectin concentrations in blood.

Interestingly, circulating levels of TNFα also rose during hyperinsulinemia, but only in the group of lean, young individuals (YS). Based on the present results, it is not possible to conclude whether BMI, age, or any other specific factor is critical for this difference between groups. It appears likely, however, that obesity attenuates the TNFα response to insulin similar to what we found with respect to IL-6. As also shown before [10], adipose tissue TNFα mRNA rose in young subjects during hyperinsulinemia, suggesting that enhanced gene expression and TNFα synthesis in adipose tissue might contribute to the rise in circulating concentrations. It has previously been suggested that adipose tissue TNFα mediates up-regulation of adipose tissue IL-6 gene expression [27]. We did not, however, detect any effect of insulin on the gene expression of IL-6 in adipose tissue; but it should be appreciated that only a limited subset from each group of subjects could be analyzed.

Our findings are in agreement with previously presented data on the effect of a hyperinsulinemic euglycemic clamp on plasma concentrations of IL-6 [8-10]. Similar to Carey et al [8] and Esteve et al [28], we demonstrate that baseline IL-6 is linked with measures of obesity. In addition, we show that the insulin-induced rise in IL-6 was negatively correlated with BMI and fasting insulin levels. There were however no significant correlations between the insulin-induced increase in IL-6 and whole-body insulin sensitivity. Nonetheless, insulin action on glucose uptake in isolated adipocytes tended to correlate positively to fold increase of IL 6; and DS as well as CS displayed a smaller relative increase of IL-6 during hyperinsulinemia compared with YS. We only identified 1 factor that independently predicted the IL-6 response to insulin; and this was BMI, which displayed an inverse relationship to IL-6 rise. Taken together, these findings are somewhat surprising. One possible interpretation is that obesity with accompanying insulin resistance leads to hyperinsulinemia, which in turn helps promote a sustained elevation of IL-6. This increase in basal IL-6 levels may result in a smaller relative rise in IL-6 upon further elevation of insulin levels, as seen in the present study.

Another possibility is that the degree of physical activity plays a role. Thus, the marked incremental effect exerted by insulin on IL-6, as well as TNFα, levels in YS could potentially be linked to a relatively high level of physical activity in this group compared with that of CS and DS. However, we did not address habits of physical activity in the study groups; and its impact vis-à-vis cytokine regulation needs further investigation.

Previous studies have shown an increase of IL-6 mRNA in adipose tissue by insulin stimulation. Adipose tissue IL-6 mRNA levels in the present study showed no significant changes during the clamp. The number of samples is small, and these results should be confirmed. However, our results would suggest other sources than adipose tissue to be involved in the insulin-stimulated increase of plasma IL-6 concentrations.

Carey et al [29] have shown that in insulin-resistant rat skeletal muscle that IL-6 gene expression is increased during a euglycemic hyperinsulinemic clamp, despite the animals being rendered insulin resistant by phosphoenolpyruvate carboxykinase (PEPCK) overexpression. They argue that this is because control of IL-6 mRNA expression is exerted via pathways that are independent of insulin resistance. Another explanation could be that the insulin-mediated increase in IL-6 levels origins from stromal-vascular cells, for example, macrophages, within adipose tissue. Further work is required to definitively show whether the rise in IL-6 in response to insulin in man is derived from skeletal muscle, adipose, or other tissue types.

There is substantial evidence [30-32] that circulating IL-6 follows a circadian rhythm; and it includes 2 troughs, one starting at around 8:00 AM and another at around 9:00 PM, as well as 2 peaks, one at around 5:00 AM and another at around 7:00 PM. Interleukin-6 concentrations remain low and relatively constant during the morning period, from 8:00 AM to 12:00 noon. Our finding of an increase in IL-6 after hyperinsulinemia during this period is therefore not likely to be caused by the circadian variation of IL-6. It should rather represent a true effect of the infused insulin.

In conclusion, acute hyperinsulinemia can produce an elevation of circulating IL-6 and, possibly, TNFα levels. The IL-6 response to insulin becomes impaired with increasing BMI, but it is not directly affected by T2DM or insulin resistance. In this study, IL-6 gene expression in adipose tissue was unaltered by insulin, whereas TNFα gene expression appeared to be increased. Given the link between the adipokines IL-6 and TNFα and cardiovascular and metabolic disease, future work is warranted to understand whether adipose tissue is involved in the production of inflammatory mediators in response to hyperinsulinemia. Elucidation of these processes will improve our understanding of the interplay between obesity, T2DM, and inflammation.

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