Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro

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[1]*Copenhagen Muscle Research Centre and* [2]*Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Denmark;* [3]*Skeletal Muscle Research Laboratory, Center for Nutrition Metabolism and Endocrinology, Royal Melbourne Institute of Technology University, Bundoora;* [4]*St. Vincent's Institute of Medical Research, University of Melbourne, Fitzroy; and* [5]*Commonwealth Scientific and Industrial Organisation Health Sciences and Nutrition, Parkville, Australia*

Petersen, E. W., A. L. Carey, M. Sacchetti, G. R. Steinberg, S. L. Macaulay, M. A. Febbraio, and B. K. Pedersen. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. *Am J Physiol Endocrinol Metab* 288: E155–E162, 2005. First published September 21, 2004; doi:10.1152/ajpendo.00257.2004.—To determine whether IL-6 increases lipolysis and fat oxidation in patients with type 2 diabetes and/or whether it exerts this effect independently of changes to the hormonal milieu, patients with type 2 diabetes (D) and healthy control subjects (CON) underwent recombinant human (rh)IL-6 infusion for 3 h. Rates of appearance (R_a) and disappearance (R_d) of [U-^{13}C]palmitate and [6,6-^{2}H$_2$]glucose were determined. rhIL-6 infusion increased ($P <$ 0.05) palmitate R_a and R_d in a similar fashion in both groups. Neither plasma glucose concentration nor glucose R_a/R_d was affected by rhIL-6 infusion in either group, whereas rhIL-6 infusion resulted in a reduction ($P < 0.05$) in circulating insulin in D. Plasma growth hormone (GH) was increased ($P < 0.05$) by IL-6 in CON, and cortisol increased ($P < 0.05$) in response to IL-6 in both groups. To determine whether IL-6 was exerting its effect directly or through activation of these hormones, we performed cell culture experiments. Fully differentiated 3T3-L1 adipocytes were treated with PBS (control) IL-6, or IL-6 plus dexamethasone and GH. IL-6 treatment alone increased ($P < 0.05$) lipolysis, but this effect was reduced by the addition of dexamethasone and GH such that IL-6 plus dexamethasone and GH had blunted ($P < 0.05$) lipolysis compared with IL-6 alone. To assess whether IL-6 increases fat oxidation, L6 myotubes were treated with PBS (Control), IL-6, or AICAR, a compound known to increase lipid oxidation. Both IL-6 and AICAR markedly increased ($P < 0.05$) oxidation of [^{14}C]palmitate compared with Control. Acute IL-6 treatment increased fatty acid turnover in D patients as well as healthy CON subjects. Moreover, IL-6 appears to be activating lipolysis independently of elevations in GH and/or cortisol and appears to be a potent catalyst for fat oxidation in muscle cells.

cytokines; metabolism; insulin sensitivity

THE CYTOKINE INTERLEUKIN-6 (IL-6) has traditionally been known for its immunomodulatory effects, but recent research has focused on its metabolic role in the etiology of obesity and type 2 diabetes. Studies have demonstrated that acute IL-6 administration can alter fat metabolism and lipolysis. We (28) and others (13) have demonstrated that the infusion of recombinant human (rh)IL-6 into healthy humans increases whole body lipolysis and free fatty acid (FFA) oxidation. In addition, Wallenius et al. (30) recently demonstrated that an IL-6 knock-out mouse became obese, a phenotype which was partially reduced with IL-6 treatment. Hence, it has been suggested that IL-6 is a possible target therapy for obesity-related disorders (30). In line with altered fatty acid metabolism, the effect of IL-6 on insulin sensitivity is not completely clear, since a number of both in vitro and in vivo studies have demonstrated that IL-6 decreases (3, 10, 11, 18, 20), increases (25, 26, 30), or has no effect (23) on insulin sensitivity.

Obesity is currently reaching epidemic proportions in western society and is recognized as a major contributing factor toward the development of type 2 diabetes. Patients with type 2 diabetes have dramatically altered metabolism, whereby they have reduced ability to oxidize fatty acids in the basal state, and upon insulin stimulation are unable to reduce fatty acid oxidation in preference for metabolism of glucose (for review see Ref. 9). Consequently, if IL-6 is proposed as a potential treatment for obesity-related disorders (3), further studies in patients with type 2 diabetes are warranted. The first aim of our study was, therefore, to examine whether the effect of IL-6 on lipid oxidation found in healthy subjects could be maintained in patients with type 2 diabetes. We hypothesized that the lipolytic effects seen in healthy young men (28) would also be prevalent in older subjects with and without type 2 diabetes.

It is apparent that elevated FFA and augmented FFA oxidation may result in transient insulin resistance (for review see Ref. 12). Although we have previously shown unaltered glucose turnover in response to IL-6 in healthy humans (23), an augmented FFA turnover may explain a mechanism by which elevated circulating IL-6 concentrations could induce transient insulin resistance in vivo. Therefore, we attempted to ascertain whether the infusion of IL-6 would alter basal glucose homeostasis and insulin concentrations concomitantly with measures of fatty acid turnover.

In previous studies that have examined the effects of infusion of high physiological doses of IL-6 on fatty acid and glucose metabolism (23, 28), circulating cortisol levels were elevated to levels that might independently affect fatty acid and glucose metabolism. Furthermore, it was proposed that the lipolytic effects seen with rhIL-6 infusion could have been the result of an IL-6-induced release of growth hormone (8). In previous rhIL-6 infusion studies, growth hormone was not measured (13, 23, 28). Because IL-6 may alter the circulating levels of key lipolytic hormones, it is difficult to determine

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whether IL-6 can act directly on lipolytic processes or act via its influence on the hormonal milieu. Therefore, a further major aim of this study was to analyze the effects of IL-6 alone and in combination with these lipolytic regulatory hormones on lipolysis in cultured adipocytes and also to determine whether IL-6 can independently increase fatty acid oxidation in cultured myotubes. We hypothesized that IL-6 would increase lipolysis and fat oxidation in an independent manner.

METHODS

Subjects

Nine elderly male patients with type 2 diabetes [D; age 67.4 ± 3.6 yr, weight 86.9 ± 6.8 kg, body mass index (BMI) 27.3 ± 1.7 kg/m²] and six healthy male controls (CON), who were statistically similar in age, weight, and BMI (age 63.8 ± 3.4 yr, weight 88.6 ± 2.0 kg, BMI 28.3 ± 1.1 kg/m²) were recruited for this study, which was approved by the Ethics Committee of Copenhagen and Frederiksberg Communities, Denmark, and performed according to the Declaration of Helsinki. The categorization of patients to group D was determined by a fasting blood glucose concentration >7.0 mmol/l, and >11.1 mmol/l after an oral glucose tolerance test, which was according to World Health Organization 1999 criteria. None of the patients received insulin treatment, nor were any of them taking oral hypoglycemic agents or other medication known to alter carbohydrate metabolism. Subjects were informed about the possible risks and discomfort involved before giving their written consent to participate. Heart rate and body temperatures for all subjects were monitored regularly throughout the experiments.

Protocol

Subjects reported to the laboratory at 0800 after an overnight fast. They visited the laboratory on two occasions separated by 7–14 days. On one occasion, they were infused with rhIL-6 (IL-6), and on the other occasion isotonic saline (SALINE) (both mixed in sterile human albumin). Subjects voided, changed into appropriate hospital attire, and remained supine during the entire experiment. They were permitted to consume only water ad libitum during the experiment. Once they were resting in a supine position, a forearm vein was cannulated for blood sampling. Two veins on the opposing arm were then cannulated for infusion of stable isotopes and IL-6/SALINE. Immediately after a blood sample was obtained for background enrichment, plasma hormones, metabolites, and cytokines, a primed (D, 36 μmol/kg; CON, 17.6 μmol/kg) continuous infusion of [6,6-²H₂]glucose (0.3 μmol·kg⁻¹·min⁻¹) was started along with a constant infusion of [U-¹³C]palmitate (0.015 μmol·kg⁻¹·min⁻¹). All isotopes were purchased from Cambridge Isotope Laboratories (Andover, MD). Two hours later, a constant-rate infusion of rhIL-6 or SALINE was begun and continued for 3 h. Infusion of stable isotopes and blood collection continued for a further 2 h post-rhIL-6/SALINE infusion. Blood samples were obtained at rest and every 60 min during a 3-h infusion as well as every 60 min during a 2-h recovery period. In addition, blood samples were obtained 30, 15, and 0 min before the commencement of infusion to ensure adequate tracer equilibration. Both [6,6-²H₂]glucose and [U-¹³C]palmitate indeed reached steady state before the commencement of infusion (data not shown). Blood samples were immediately spun, and plasma was taken and stored at −80°C until further analysis of palmitate and glucose enrichments, as well as concentrations of fatty acids (FA), triglycerides, palmitate, glycerol, glucose, insulin, glucagon, cortisol, IL-6, TNF-α, epinephrine, and norepinephrine. The amount of blood taken during each experiment was 180 ml.

IL-6 Infusion

Previous studies from our laboratory (23, 28) revealed that administration of a specific dose of rhIL-6 results in circulating concentrations of ∼140 pg/ml. This dose is similar to circulating IL-6 levels that may be found after very strenuous physical exertion, such as a marathon run (22). This dose has previously been shown to have no effect on circulating epinephrine or norepinephrine concentrations (28). Our pilot work and data from these studies demonstrated that IL-6 delivered in 2% human albumin at a rate of 7 μg/h would result in approximately this concentration (140 pg/ml); therefore, this was the amount administered to all subjects during IL-6 treatment. Subjects were randomly assigned and blinded to SALINE and IL-6 treatments.

Analyses

Plasma palmitate concentration was determined by GC (Autosystem XL, Perkin Elmer), using heptadecanoic acid as internal standard, and plasma [U-¹³C]palmitate enrichment was determined by GC-combustion isotope ratio mass spectrometry (GC-C-IRMS; Hewlett-Packard 5890-Finnigan GC combustion III-Finnigan Deltaplus, Finnigan MAT, Germany). In preparation of GC and GC-C-IRMS analysis, plasma samples were processed to make a methyl derivative of palmitate. Briefly, heptadecanoic acid (30 nmol) was added, as an internal standard, to 200 μl of plasma, and the proteins were precipitated with ice-cold acetone. After centrifugation, lipids were extracted with hexane. The plasma tri-, di-, and monoacylglycerols, phospholipids, cholesterol, and FFAs were isolated by thin-layer chromatography (petroleum ether-diethyl ether-acetic acid, 120:25:1.5, vol/vol/vol) on silica gel 60 plates (Merck, Darmstadt, Germany). After development of the plates the FFA band was isolated, and 2 ml of methanol and isooctane (4:1 vol/vol), and 0.2 ml of acetyl chloride were added and heated for 1 h at 100°C. Thereafter, 5 ml of 6% potassium carbonate were added and mixed. After centrifugation, the upper layer was evaporated under N₂ and redissolved in isooctane. The palmitate concentration was determined by injecting 2 μl in the split mode (1/5), onto a 30-m capillary fused-silica column (Rtx-2330, Restex), injector temperature at 300°C. Helium carrier gas was used at a flow rate of 1.8 ml/min. The instrument was controlled and the palmitate concentration automatically calculated from a palmitic and heptadecanoic acid standard curve. The palmitate enrichment was determined by injecting 2 μl onto a 30-m capillary fused-silica column (Rtx-2330, Restex) via an HP-PTV injector. The vent flow of the injector was set at 5 psi, and the initial temperature was set at 85°C for 1 min and then increased with a ramp of 500°C/min to 255°C. The isotopic enrichment of palmitate was expressed as the difference (Δ) between the ¹³C-to-¹²C ratio of the sample and a known laboratory reference standard related to Pee Dee Belemnitella (PDB) limestone. The methyl derivative of palmitate contains 17 carbons, of which 16 are palmitate; thus the tracer-to-tracee ratio (TTR) of palmitate was corrected by a factor 17/16. Finally, whole body palmitate rates of appearance (R$_a$) and disappearance (R$_d$) were determined using previously described calculations (27).

Plasma [6,6-²H₂]glucose enrichment was determined as follows: 250 μl of water and 3 ml of chloroform-methanol (2.3:1, vol/vol) were added to 150 μl of plasma, mixed, and centrifuged at 4°C for 15 min. The supernatant was decanted and washed once by adding 1 ml of water (pH 2 with hydrochloric acid) and 2 ml of chloroform before being spun as described above. The upper layer was dehydrated and derivatized with the addition of butylboronic acid and pyridine (100 mg:10 ml, wt/vol) and incubated at 95°C for 30 min. Thereafter, 250 μl of acetic anhydride were added and incubated at room temperature for 90 min. The solution was dehydrated and redissolved in 100 μl of ethyl acetate. The deuterium enrichment of glucose was determined by split injection (ratio 1:30) of 1-μl samples by use of a gas chromatograph-mass spectrometer (GC column, CP-SIL 8CB, Chromopack, The Netherlands). Glucose R$_a$ and R$_d$ were determined from

changes in the percent enrichment in the plasma of $[6,6-^2H_2]$glucose, calculated using the one-pool non-steady-state model (Steele et al. 1956), assuming a pool fraction of 0.65 and estimating the apparent glucose space as 25% of body weight. The metabolic clearance rate (MCR) of glucose, which represents the amount of plasma required to clear a set amount of glucose, was calculated by dividing glucose R_d by the plasma glucose concentration.

Plasma was also analyzed enzymatically for concentrations of metabolites of glycerol, triacylglycerol (TAG), FA, and glucose on an automatic analyzer (Cobas Fara, Roche, Switzerland). Plasma IL-6 and TNF-α were measured by high-sensitivity ELISA, with inter- and intra-assay coefficients of variation, as specified by the manufacturer, of 7.4 and 7.8% (IL-6) and 6.7 and 13.3% (TNF-α), respectively (R&D Systems, Minneapolis, MN). Plasma insulin (Insulin RIA 100; Amersham, Pharmacia, Biotech, Uppsala, Sweden), glucagon (Linco Research, St. Charles, MO), growth hormone (Diagnostic Products, Los Angeles, CA) and cortisol (Diagnostic Products) were analyzed by radioimmunoassay and plasma epinephrine and norepinephrine by high-performance liquid chromatography according to Hjemdahl et al. (6).

Cell Culture Experiments

Lipolysis. 3T3-L1 fibroblasts were grown to confluence in DMEM-F12 in 5% FBS. Medium was removed, and differentiation medium added [DMEM-F-12 in 5% FBS containing Actrapid insulin (0.5 mU/ml; Novo Nordisk), 0.1 µg/ml dexamethasone, and 25 µg/ml IBMX]. After 3 nights, differentiating medium was removed and replaced with DMEM-F-12 in 5% FBS with 0.5mU/ml Actrapid insulin. The medium was then replaced every 2 days until lipid droplets were present in cells, at which time experiments were completed. To assess lipolytic effects of IL-6, DMEM with 0.1% FBS was added to differentiated 3T3-L1 adipocytes overnight to serum starve the cells. The following morning, fresh low-serum (0.1%) medium was added, and experiments were performed by treating cells for 120 min with 20 ml of PBS (CON), 100 ng/ml IL-6 (IL-6), or 100 ng/ml IL-6 with the addition of 0.1 ug/ml dexamethasone (Sigma-Aldrich, Castle Hill, Australia) and 10 ng/ml growth hormone (Genotropin; Pharmacia). The concentrations of these latter treatments were based on previous studies (1, 31). Medium was collected from triplicate cell incubations and frozen for subsequent glycerol analysis, using an enzymatic assay linked to NADH with fluorometric detection. Briefly, lysates were thawed and diluted 1:10 in sterile water; 20 µl of dilute sample were added to 1 ml of buffer containing 1 M hydrazine, 0.2 M glycine, 1 mM EDTA, 2 mM $MgCl_2$, 0.2 mM NAD^+, and 0.5 mM ATP. Fluorescence was determined via measurement of sample at an excitation gamma of 365 nm and emission gamma of 455 nm to determine fluorescence of NADH, using a Jasco FP-750 Spectrofluorometer (Padbury, Australia). The reaction was then initiated through addition of 50 µl of dilute enzymes of glycerol kinase (0.25 U/ml) and glycerophosphate dehydrogenase (2 U/ml) and allowed to proceed for 60 min in the dark. This results in conversion of glycerol to glycerol phosphate, and this reacts with NAD^+ to form dihydroxyacetone phosphate and NADH. The fluorescence emitted by NADH was again determined, and glycerol concentration was calculated as the difference in fluorescence before and after addition of enzymes, compared with predetermined glycerol standards (10 to 800 µM). All reagents were obtained from Sigma-Aldrich.

Fat oxidation. L6 myoblasts were maintained at 37°C on 100-mm collagen-coated plastic dishes in 5% CO_2-95% O_2 humidified air in α-MEM plus 10% fetal bovine serum (FBS) culture medium. Differentiation was induced by switching to medium containing 2% FBS when the myoblasts were ~90% confluent. Experimental treatments commenced after 7 days, by which time nearly all of the myoblasts had fused to form myotubes. The evening before experiments, cells were serum starved in α-MEM plus 0.1% FBS. Experiments were performed the following morning in α-MEM media plus 0.1% FBS

containing 4% FA-free bovine serum albumin, 1.0 mmol/l palmitate, and 2 µCi of $[1-^{14}C]$palmitate and were treated for 120 min with 20 ml of PBS (CON), 2 mmol/l 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), or 100 ng/ml IL-6. After 2 h, 1.0 ml of incubation medium was added to a 20-ml glass scintillation vial containing 1.0 ml of 1 M H_2SO_4 and a 0.5-ml microcentrifuge tube containing 1 M benzethonium hydroxide. Liberated $^{14}CO_2$ was trapped in the benzethonium hydroxide over 60 min, and the microcentrifuge tube containing trapped $^{14}CO_2$ was placed in a scintillation vial and counted.

Statistics

All data are presented as means $\pm$ SE (D, $n = 9$; CON, $n = 6$). Group physical characteristics were analyzed using a one-way analysis of variance (ANOVA). To analyze changes over time, treatments, and the different groups, a three-way repeated-measures ANOVA was used for plasma hormones and metabolites and data for glucose and palmitate R_a and R_d. Newman-Keuls post hoc tests were used where significant differences from ANOVA were revealed. Significance was accepted as $P < 0.05$. Because the number of conditions between treatments differed in the cell culture experiments, data were analyzed using multiple t-tests with Bonferroni correction. All statistics were completed using Statistica software for Windows (StatSoft, v. 5.1, 1997; Statistica, Tulsa, OK).

RESULTS

D and CON were not different for age, weight, and BMI ($P > 0.05$). Neither heart rate nor body temperature was affected by time or treatment in either group (data not shown). Importantly, as in our previous experiments in healthy young humans (21, 28), rhIL-6 infusion had no adverse side effects.

Plasma Cytokines

rhIL-6 infusion increased ($P < 0.05$) plasma IL-6 concentrations to ~200 pg/ml in both groups (Table 1). During saline infusions, plasma IL-6 concentrations progressively rose, such that they were elevated ($P < 0.05$) at the end of the experiments (Table 1). This was presumably the result of prolonged fasting, and there were no differences when groups were compared in either trial. Plasma TNF-α was not affected by time, treatment or group (Table 1). Although the IL-6 concentrations resulting from infusions were higher than we had predicted, patients felt no ill effects and therefore remained blinded to treatments throughout the studies.

Table 1. *Plasma cytokine concentrations*

	Pre	Infusion 60 min	Infusion 180 min	120 min Post
IL-6, pg/ml				
D IL-6	7.5±2.1	181±35*†	204±31*†	16±3.7*†
D SALINE	4.3±0.7	5.8±1.0	6.6±0.9	8.3±1.1*
CON IL-6	5.1±1.5	199±71*†	220±60*†	11±2.0*†
CON SALINE	2.8±0.2	4.5±0.8	5.2±0.4	6.5±0.8*
TNF-α (pg/ml)				
D IL-6	2.8±0.50	NM	2.5±0.37	2.6±0.38
D SALINE	2.5±0.40	NM	2.6±0.36	2.7±0.40
CON IL-6	2.2±0.36	NM	2.1±0.33	2.0±0.34
CON SALINE	2.1±0.26	NM	2.2±0.36	2.2±0.29

Values are means $\pm$ SE. NM, not measured. *Difference ($P < 0.05$) from Pre; †difference ($P < 0.05$) from SALINE.

Plasma Hormones

Plasma catecholamines, cortisol, glucagon, and growth hormone levels are presented in Table 2. Plasma epinephrine concentrations were unaffected by time or treatment. In contrast, although norepinephrine concentrations were not significantly altered by IL-6 infusion in either group, there appeared to be an initial, but not significant, rise in the concentration of this neurotransmitter after 60 min of infusion when IL-6 was compared with saline (CON, $P = 0.4$; D, $P = 0.1$), after which concentrations gradually returned to approximately preinfusion levels. Plasma cortisol and glucagon levels were also elevated ($P < 0.05$) after 60 min of rhIL-6 infusion and remained elevated ($P < 0.05$) until 120 min post- and 60 min postinfusion, respectively. Of note, there were no differences in any of these hormone concentrations when D was compared with CON. Plasma growth hormone was elevated ($P < 0.05$) after 60 min of infusion in IL-6 compared with Saline in CON. After this time, circulating growth hormone returned to basal levels. Of note, growth hormone was not significantly affected by rhIL-6 infusion in D. This was not due to the fact that growth hormone follows circadian release patterns, since all patients begun infusions at the same time of day following an overnight fast.

Isotopic Tracer-Determined Glucose and FFA Kinetics

Plasma glucose concentrations were higher ($P < 0.05$) in D compared with CON. However, tracer-determined rates of neither glucose R_a nor R_d were different between groups (Fig. 1). In addition, tracer-determined rates of glucose R_a and R_d were not altered in either group with rhIL-6 infusion (Fig. 1). No differences in MCR were found between groups or as a result of rhIL-6 infusion (data not shown). Plasma FFA tended to be higher in D compared with CON at rest, but results were not significant. However, rhIL-6 infusion markedly increased ($P < 0.05$) plasma FFA at 180 min and thereafter (Fig. 2) in both D and CON. In addition, rhIL-6 infusion increased ($P < 0.05$) isotopic tracer-determined rates of palmitate R_a and R_d (Fig. 2) in both subject groups.

Plasma TAG and Glycerol Concentrations

Plasma TAG concentrations were unaffected by time or treatment (Fig. 3). In addition, there were no differences in plasma TAG when D was compared with CON. Likewise, plasma glycerol followed the same pattern, although there was a trend ($P = 0.09$) for plasma glycerol to be elevated with rhIL-6 infusion (Fig. 3).

Plasma Insulin

Plasma insulin was higher ($P < 0.05$) in D compared with CON. In addition, plasma insulin was lower ($P < 0.05$) after 120 min in both CON and D (time by treatment interaction; Fig. 4).

Cell Culture Experiments

Glycerol in the medium from the IL-6-treated 3T3-L1 cells was higher ($P < 0.05$) compared with Control, whereas the addition of growth hormone and dexamethasone reduced ($P < 0.05$) glycerol compared with IL-6 alone (Fig. 5). IL-6 increased ($P < 0.05$) [^{14}C]palmitate oxidation in L6 myotubes compared with PBS-treated cells (Control). We used AICAR as a positive control because AICAR is known to increase palmitate oxidation via phosphorylation of acetyl-CoA carboxylase and to reduce malonyl-CoA (24). At the concentrations used in this experiment, IL-6 stimulated palmitate oxidation to a greater extent ($P < 0.05$) than AICAR (Fig. 5).

Table 2. *Plasma hormone concentrations.*

	Pre	60 min	120 min	180 min	60 min Post	120 min Post
Epinephrine, nmol/l						
D IL-6	0.36±0.10	0.23±0.06	0.45±0.17	0.21±0.03	0.28±0.06	0.35±0.16
D SALINE	0.54±0.19	0.79±0.35	0.27±0.06	0.45±0.08	0.30±0.11	0.50±0.19
CON IL-6	0.47±0.22	0.53±0.18	0.15±0.05	0.26±0.10	0.29±0.14	0.53±0.13
CON SALINE	0.37±0.14	0.17±0.04	0.13±0.02	0.27±0.15	0.13±0.02	0.23±0.11
Norepinephrine, nmol/l						
D IL-6	1.0±0.10	1.7±0.30	1.3±0.17	1.27±0.21	1.2±0.20	1.19±0.16
D SALINE	1.2±0.21	1.0±0.27	0.75±0.11	0.8±0.14	0.81±0.08	1.00±0.13
CON IL-6	1.2±0.84	2.0±0.59	1.7±0.35	1.3±0.22	1.4±0.40	1.38±0.3
CON SALINE	1.7±0.62	1.3±0.39	0.95±0.33	1.3±0.32	1.4±0.27	1.25±0.24
Cortisol, pg/ml						
D IL-6	6.6±0.96	16±1.3*	22±1.1*	16±0.7*	11±0.78*	8.62±0.76
D SALINE	10.2±1.8	7.7±1.6	8.3±0.97	8.1±0.92	6.8±1.1	6.95±1.04
CON IL-6	8.1±1.9	18±1.6*	27±3.7*	18±3.0*	13±2.3*	9.95±1.62
CON SALINE	9.1±0.99	5.7±0.60	5.9±1.1	5.6±0.95	6.0±0.56	8.24±1.97
Glucagon, pg/ml						
D IL-6	74±7.5	80±7.3*	93±7.3*	87±7.4*	87±9.2*	86.2±7.9
D SALINE	70±9.0	72±7.9	72±10	66±7.3	68±8.2	68.2±8.6
CON IL-6	76±11	85±16*	92±15*	85±12*	81±12*	91.5±15.6
CON SALINE	74±8.7	71±9.9	69±10	74±11	74±11	84.2±16.8
Growth hormone, ng/ml						
D IL-6	0.29±0.05	1.2±0.41		0.76±0.16		0.36±0.12
D SALINE	0.22±0.06	0.67±0.32		0.84±0.33		0.56±0.15
CON IL-6	0.91±0.38	3.2±0.79*		0.46±0.09		1.73±0.98
CON SALINE	0.75±0.28	0.20±0.06		1.3±0.40		0.69±0.22

*IL-6 trial different from saline trial; †group main affect, D different from CON
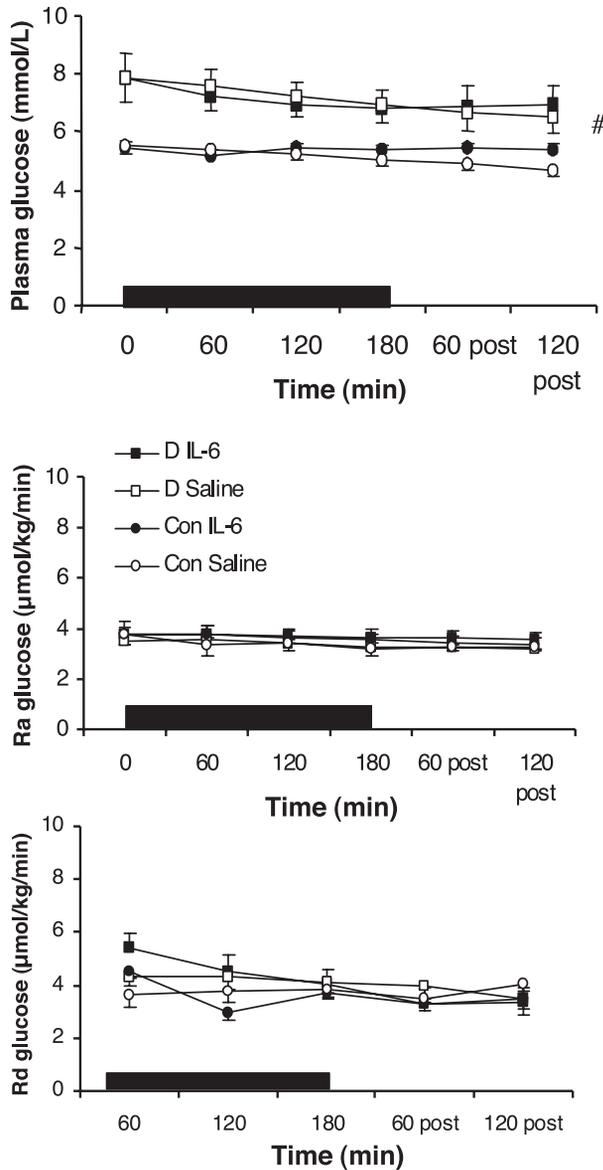
Fig. 1. Plasma glucose (*top*), glucose rate of appearance (R_a; *middle*) and glucose rate of disappearance (R_d; *bottom*) before (0 min), during, and after IL-6 or saline infusion in patients with type 2 diabetes (D) or age-matched control subjects (CON). Filled bar, infusion. #Main group effect (D > CON, $P < 0.05$).

The marked increase in lipolysis and palmitate R_d with IL-6 infusion (Fig. 2) support previous observations by us (28) and others (13). The mechanism/s by which IL-6 may exert its effect on adipocytes is/are not completely clear. In the present study, we saw an increase in cortisol, glucagon, and growth hormone (Table 2) and, therefore, our in vivo human experiments cannot rule out the possibility that the lipolytic action of IL-6 is via modulation of these hormones. Indeed, Jensen (8)
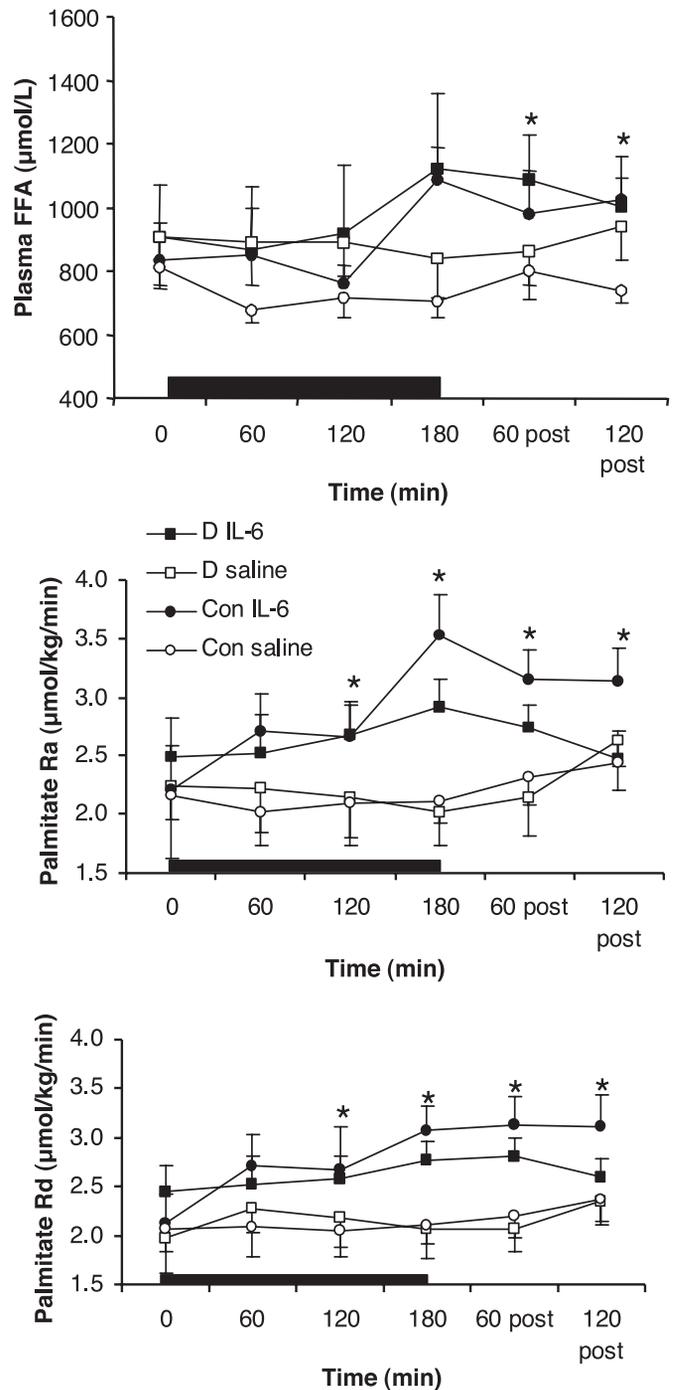
Fig. 2. Plasma free fatty acids (FFA; *top*), palmitate R_a (*middle*) and palmitate R_d (*bottom*) before (0 min), during, and after IL-6 or saline infusion in D or CON. Filled bar, infusion. *Difference between IL-6 and saline (treatment × time interaction).

DISCUSSION

Our data demonstrate that rhIL-6 infusion is capable of increasing fatty acid turnover in elderly subjects with or without type 2 diabetes without affecting insulin sensitivity. Moreover, our cell culture experiments indicate that IL-6 alone markedly increases both lipolysis and fat oxidation, and alterations in the hormonal milieu are not additive to the lipolytic effects of IL-6. To our knowledge, these are the first reported data to show such effects. Therefore these data provide new insights into the effect of IL-6 on fat metabolism in patients with type 2 diabetes and suggest that IL-6 can act independently of lipolytic hormones and provide preliminary evidence that IL-6 may be a viable therapeutic treatment for obesity and its complications, such as type 2 diabetes.

Fig. 3. Plasma triacylglererol (*top*), and glycerol (*bottom*) before (0 min), during, and after IL-6 or saline infusion in D or CON. Filled bar, infusion.
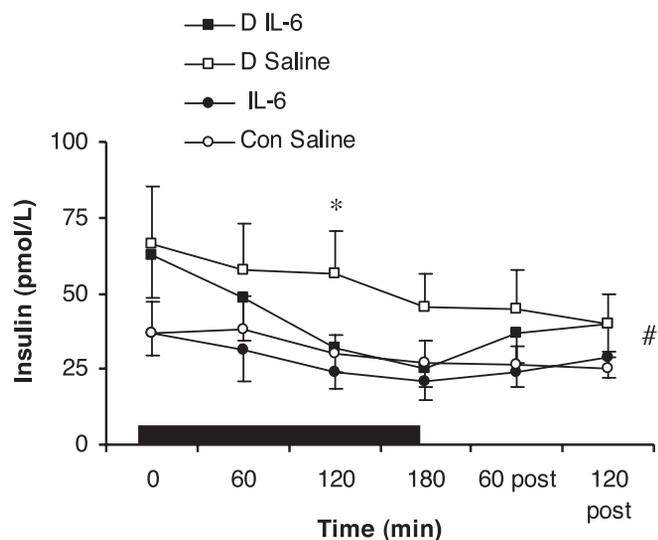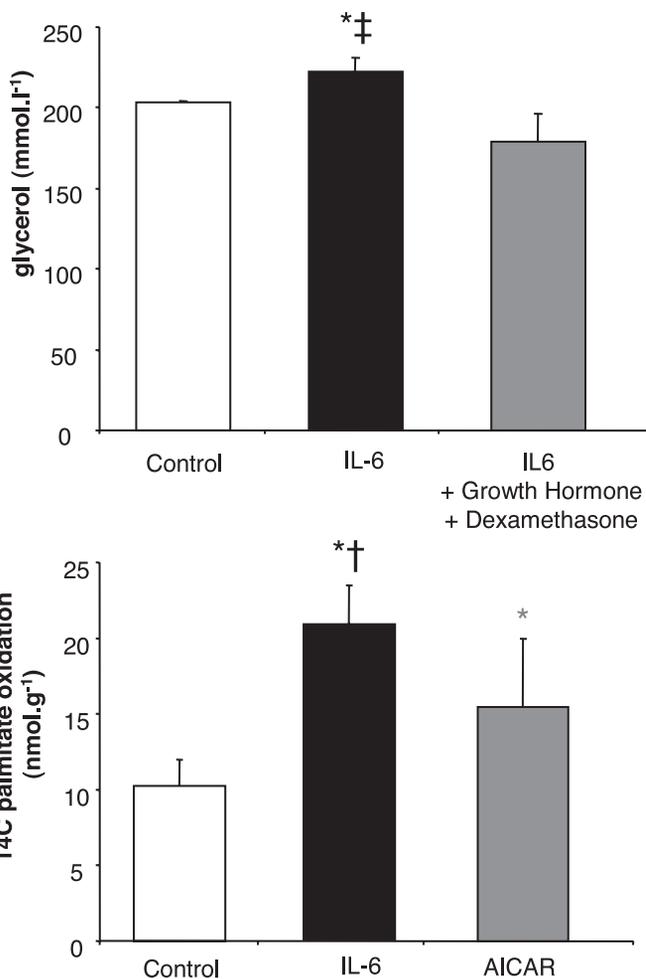
Fig. 5. Plasma glycerol release from 3T3-L1 adipocytes treated with PBS (control), IL-6, or IL-6 combined with dexamethasone and growth hormone (*top*) and [^{14}C]palmitate oxidation in L6 myotubes treated with PBS (control), IL-6, or 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR; *bottom*). *Difference ($P < 0.05$) compared with Control; ‡difference compared with IL-6 + growth hormone + dexamethasone; †difference ($P < 0.05$) compared with AICAR.

recently suggested that the effects of IL-6 on lipolysis that we previously observed in young healthy subjects (28) were secondary to changes in hormones. However, in our previous study (28) glucagon was not increased with rhIL-6 infusion, yet the magnitude of increase in plasma FFA and palmitate R_a/R_d was almost identical to the present data. We suggest, therefore, that the increase in glucagon may have been due to the transient decrease in plasma insulin observed in the present study (Fig. 4) and it is unlikely that glucagon plays a major role in the lipolytic action of IL-6. However, in the present study, cortisol levels were increased by IL-6 infusion while growth hormone was significantly increased in CON, but not D (Table 2). While these increases may account for the lipolytic effect of IL-6 in vivo, this appears unlikely because cortisol has been found to be anti-lipolytic in vivo (7, 15). Furthermore, growth hormone was not significantly elevated during IL-6 infusion in the patients with type 2 diabetes, but these subjects nonetheless experienced increased whole body fatty acid turnover in these circumstances. The fall in circulating insulin concentrations over time might account for the increase in lipolysis seen in

Fig. 4. Plasma insulin before (0 min), during, and after IL-6 or saline infusion in D or CON. Filled bar, infusion. #Main group effect (D > CON, $P < 0.05$); *difference between IL-6 and saline (treatment × time interaction).

both groups, however, the fall in insulin was dramatic in D and not significant in CON. Conversely, the lipolytic rates induced by IL-6 were quantitatively (although not statistically significantly) greater in CON compared with D.

We acknowledge, that we could not test the hypothesis that IL-6 acts on fat metabolism per se using an in vivo human model, particularly in light of recent evidence showing that cortisol and growth hormone alone and additively effect regional lipolysis in humans (5). Therefore, we tested the hypothesis that IL-6 acts directly on fat metabolism using tissue culture preparations. We found that glycerol release from cultured 3T3-L1 adipocytes was increased by IL-6 and that IL-6 indeed acts independently of hormonal changes because the addition of growth hormone and cortisol (dexamethasone) blunted rather than augmented the lipolytic response (Fig. 5). In addition, we demonstrated that in myotubes, IL-6 alone is able to stimulate fat oxidation (Fig. 5), although the mechanism by which this occurs remains to be elucidated. It was somewhat surprising that the addition of growth hormone and dexamethasone to IL-6 negated the lipolytic actions of IL-6 when these agents are known to induce lipolysis (8). We have no explanation for this finding, but it appears that IL-6 negated the lipolytic actions of these hormones.

Regardless of the mechanism by which IL-6 enhanced lipolysis, it is clear that both palmitate R_a and R_d were enhanced by IL-6 and, based on our previous study (28), the fatty acids taken up by tissues are oxidized at a rate that matches R_d. Importantly, the increase in fatty acid R_d and oxidation, which, based on current knowledge would be expected to impair glucose metabolism, does not appear to impair glucose oxidation since whole body glucose R_d was not impaired by IL-6 (Fig. 1). Of note, IL-6 treatment increases oxygen consumption in both mice (30) and humans (13). Taken together, the present and previous studies (13, 28, 30) suggest that IL-6 increases fatty acid metabolism and whole body energy metabolism, rather than causing an inhibition of glucose metabolism.

Increased availability and oxidation of fatty acids reduces insulin sensitivity (17). Likewise, it is well known that cortisol (16) and growth hormone (7) induce insulin resistance. In the present study, fatty acid turnover was increased and both cortisol and growth hormone (in C) were elevated with IL-6 infusion (Table 2). Given the reported therapeutic potential of IL-6 (29), it was very important to examine the effect of IL-6 on insulin levels. While inducing effects that would appear to lead to transient insulin resistance, IL-6 did not elevate insulin concentration. In fact, at 120 min of infusion, plasma insulin levels were reduced rather than elevated. Importantly, the effect in D was marked, such that these patients reached insulin levels comparable to those seen in CON after 2–3 h of IL-6 infusion (Fig. 4). Since IL-6 increases β-cell insulin production and secretion in vitro (4, 19), and because increased levels of FFA reduce insulin clearance (2), we suggest that the accentuated fall in circulating insulin levels is the result of metabolic changes other than a direct effect of IL-6 on the β-cells. Furthermore the changes in circulating insulin levels during saline infusions were similar in both subject groups, therefore altered β-cell function in the patient group is unlikely to be the reason for the fall while infused with IL-6. Although it might be somewhat preliminary to imply infusion of IL-6 improved glucose homeostasis, it can at least be concluded that IL-6 does not impair basal glucose homeostasis. It is also tempting to speculate that IL-6 might be able to improve the action of insulin in the short term, however, this can only be determined with certainty by a more valid method, such as the euglycemic-hyperinsulinemic clamp. Although previous investigations have analyzed plasma IL-6 levels and IL-6 gene polymorphisms in relation to insulin sensitivity as measured by the clamp technique (3, 14), they have not directly established whether IL-6 plays a causative role in the induction of insulin resistance.

In conclusion, we have identified a cytokine that, when infused into humans, can increase fatty acid turnover in patients with type 2 diabetes and aged-match control subjects, without reduction in glucose turnover. Importantly, this can occur without causing adverse side effects or hypertriacylglyceridemia and the actions appear independent of changes in circulating hormones. Hence, we suggest that there is potential for IL-6 to be used therapeutically in the treatment of obesity-related disorders, such as type 2 diabetes, providing long-term efficacy can be established.

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