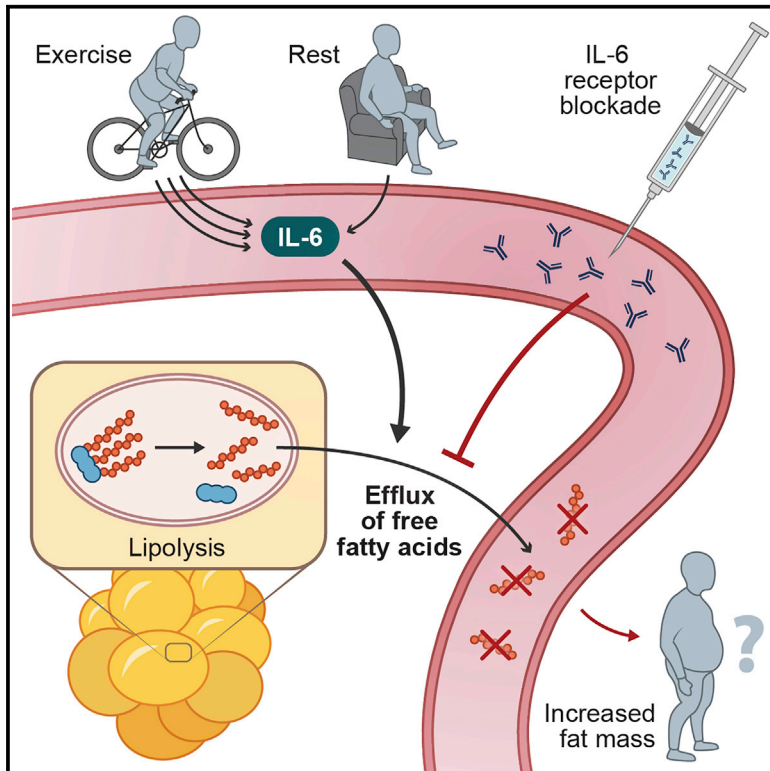


Blocking endogenous IL-6 impairs mobilization of free fatty acids during rest and exercise in lean and obese men

Graphical abstract



Authors

Beckey Trinh, Merel Peletier, Casper Simonsen, ..., Bente Klarlund Pedersen, Gerrit van Hall, Helga Ellingsgaard

Correspondence

helga.ellingsgaard@regionh.dk

In brief

Trinh et al. show that, in humans, less free fatty acids are mobilized when endogenous IL-6 signaling is blocked. Their findings highlight a possible mechanism by which treatment with IL-6 receptor blockade leads to expansion of fat mass and potentially affects the metabolic health of affected individuals.

Highlights

- IL-6 receptor blockade lowers mobilization of free fatty acids in lean and obese men
- The lowered fatty acid mobilization occurs during rest, exercise, and recovery
- IL-6 receptor blockade slightly impairs lipolysis only in obese men



Article

Blocking endogenous IL-6 impairs mobilization of free fatty acids during rest and exercise in lean and obese men

Beckey Trinh,¹ Merel Peletier,¹ Casper Simonsen,¹ Peter Plomgaard,^{1,2,3} Kristian Karstoft,^{1,4} Bente Klarlund Pedersen,¹ Gerrit van Hall,^{2,5,6} and Helga Ellingsgaard^{1,7,*}

¹The Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

²Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

³Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

⁴Department of Clinical Pharmacology, Bispebjerg-Frederiksberg Hospital, University of Copenhagen, Copenhagen, Denmark

⁵Clinical Metabolomics Core Facility, Rigshospitalet, Copenhagen, Denmark

⁶Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

⁷Lead contact

*Correspondence: helga.ellingsgaard@regionh.dk

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SUMMARY

Lack of interleukin-6 (IL-6) leads to expansion of adipose tissue mass in rodents and humans. The exact underlying mechanisms have not been identified. In this placebo-controlled, non-randomized, participant-blinded crossover study, we use the IL-6 receptor antibody tocilizumab to investigate the role of endogenous IL-6 in regulating systemic energy metabolism at rest and during exercise and recovery in lean and obese men using tracer dilution methodology. Tocilizumab reduces fatty acid appearance in the circulation under all conditions in lean and obese individuals, whereas lipolysis (the rate of glycerol appearance into the circulation) is mostly unaffected. The fact that fatty acid oxidation is unaffected by IL-6 receptor blockade suggests increased re-esterification of fatty acids. Glucose kinetics are unaffected. We find that blocking endogenous IL-6 signaling with tocilizumab impairs fat mobilization, which may contribute to expansion of adipose tissue mass and, thus, affect the health of individuals undergoing anti-IL-6 therapy (ClinicalTrials.gov: NCT03967691).

INTRODUCTION

Some 20 years ago, it was shown that circulating interleukin-6 (IL-6) levels increase not only during infection or autoinflammatory diseases but also in response to physical exercise and that production and release of IL-6 by contracting skeletal muscle largely accounts for this increase.^{1–3} Since then, infusion of recombinant human IL-6 (rhIL-6) has been used to mimic exercise-induced IL-6 to study its role in energy metabolism.^{4–6} These studies have shown that infusion of rhIL-6 into healthy humans increases lipolysis in adipose tissue and skeletal muscle and at the whole-body level. In addition to stimulating mobilization of fat from its stores, exogenous IL-6 has been found to increase fat oxidation in skeletal muscle and at the whole-body level.^{4–6}

In obese humans, circulating IL-6 levels are sometimes slightly elevated,^{7,8} and IL-6 resistance has been suggested as one possible underlying cause for the metabolic disturbances associated with obesity.^{9,10} In fact, lacking the IL-6 signal is associated with expansion of adipose tissue mass in mice and humans.^{11–13} In humans, the IL-6 receptor antibody tocilizumab can be used to block endogenous IL-6 signaling to study the

physiological role of IL-6. In a recent study, we showed that IL-6 plays a physiological role in regulating adipose tissue mass in humans;^{12,13} individuals with abdominal obesity treated with tocilizumab failed to reduce visceral and epicardial adipose tissue mass in response to exercise training. How exactly endogenous IL-6 regulates adipose tissue mass in humans is largely unknown.

In the present study, we investigated the role of endogenous IL-6 in regulating fat turnover in humans. We used the IL-6 receptor antibody tocilizumab to block endogenous IL-6 signaling in lean and obese men during rest, exercise, and recovery and used stable, isotopically labeled substrates to study fat and glucose kinetics in detail. We hypothesized that acute and long-term IL-6 receptor blockade impairs resting and, more prominently, exercise-induced lipolysis and that, because of IL-6 resistance, this effect is diminished or absent in obese people.

Given that individuals with rheumatoid arthritis¹⁴ and, in the future, possibly also those with cardiovascular disease¹⁵ will receive long-term IL-6 blockade, it is clinically relevant to fully understand how this treatment influences energy metabolism and potentially the metabolic health of these individuals.





Figure 1. Schematic overview of the study. NaCl 0.9% was infused on saline day and day 21, and tocilizumab was infused on day 1. IC, indirect calorimetry; $Watt_{max}$, peak power output; ●, blood sample; ○, breath sample.

RESULTS

Study design

This was a placebo-controlled, non-randomized, participant-blinded crossover study in lean and obese, apparently healthy male participants (ClinicalTrials.gov: NCT03967691). The IL-6 receptor antibody tocilizumab was used to block IL-6 signaling, and saline (0.9%) served as placebo. Stable isotopically labeled glycerol, palmitate, and glucose were used to study lipid and glucose kinetics at rest and during exercise and recovery in the fasted state (Figure 1). Additionally, isotopically labeled tyrosine and phenylalanine were used to study amino acid kinetics as a separate distinctive aim not covered in this paper. Participants attended three study visits; the order was not randomized because of the long elimination half-life of tocilizumab (half-life $t_{1/2}$ after first dose = 123 ± 41.5 h¹⁶). We infused saline (0.9%) during the first visit (saline day), tocilizumab a week later during the second visit (day 1), and saline (0.9%) during the third visit (day 21 after tocilizumab infusion). This allowed us to study the acute and chronic effects of IL-6 receptor blockade on day 1 and day 21, respectively.

Participants

Between July 2019 and April 2020, 26 participants were included in the study; 13 lean and 9 obese male participants completed the study. Details about the recruitment process can be found in Figure S1. Detailed inclusion and exclusion criteria are listed in the STAR Methods. Participants were matched for age and absolute maximal oxygen uptake. Baseline characteristics are given in Table 1. As per study design, the obese group had a higher body mass index (BMI), fat mass (absolute mass and percent of body weight), waist-to-height ratio, fasting insulin, insulin resistance (Homeostatic Model Assessment for Insulin Resistance, HOMA-IR), total and low-density lipoprotein LDL cholesterol, and triglyceride (TAG) levels than the lean group, whereas fasting glucose and HbA1c were similar (Table 1). No participants took any kind of lipid-lowering, glucose-lowering, or anti-inflammatory medication.

Tocilizumab increases systemic levels of IL-6

The plasma levels of IL-6 were not different in the lean and obese groups at baseline (Table 1). The low IL-6 levels in the obese group indicate a relatively healthy group with no signs of low-

grade inflammation. On the saline day, IL-6 levels increased similarly in the lean and obese groups during exercise. The median increase of plasma IL-6 levels was 5.2-fold in the lean group and 5.0-fold in the obese group (Figure 2).

IL-6 levels increase in response to tocilizumab as a result of impaired receptor-mediated clearance of IL-6.^{17,18} As expected, infusion of tocilizumab on day 1 immediately increased IL-6 levels similarly in both groups (Figure 2), and in line with the long elimination time of tocilizumab,¹⁶ IL-6 levels remained elevated on day 21. Complete blockade of IL-6 receptors occurs when the concentration of tocilizumab is $1 \mu\text{g/mL}$.¹⁷ We did not measure plasma levels of tocilizumab; however, the single infusion of tocilizumab (dose, 8 mg/kg) used in our study is expected to lead to plasma concentrations of tocilizumab of $166 \mu\text{g/mL}$ after 2 h¹⁹ and $10 \mu\text{g/mL}$ after 21 days. Accordingly, the effects of tocilizumab on day 1 (90 min onward) and day 21 can be considered acute and chronic effects of IL-6 receptor blockade.

Relative exercise intensity in the lean and obese groups was similar

The mean power output during the exercise bout was 121 ± 4 W in the lean group and 110 ± 9 W in the obese group, corresponding to $58\% \pm 1.5\%$ and $52\% \pm 3.8\%$ of peak oxygen uptake (VO_{2peak}), respectively. The exercise intensity and perceived exertion determined using the Borg scale (12.9 ± 0.2 in the lean group and 13.2 ± 0.2 in the obese group) did not significantly differ between groups. Furthermore, IL-6 receptor blockade had no effect on perceived exertion.

IL-6 receptor blockade reduces the appearance of free fatty acids in the circulation

Plasma concentrations of palmitate rose steadily during exercise and early recovery and dropped rapidly thereafter (Figure 3A). IL-6 receptor blockade seemingly reduced palmitate concentrations with exercise only in the lean group on day 1, but this was only significant at the onset of exercise. The difference in effect between groups was not significant.

The rate of appearance of palmitate (R_a palmitate) represents the efflux of free fatty acids liberated during lipolysis. On all study visits and as normally expected, R_a palmitate increased continuously during exercise and decreased rapidly in recovery (Figures 3B and 3C). The exercise-induced median increase of R_a palmitate on the saline day was 2.3-fold in the lean group and

Table 1. Study participant characteristics

	Lean (n = 13)	Obese (n = 9)	p Value
Age (years)	26 ± 4	29 ± 3	0.0622
Body composition			
Body weight (kg)	78.7 ± 6.9	115.2 ± 9.1	0.0000
BMI (kg/m ²)	23.1 ± 1.2	34.3 ± 2.8	0.0000
Hip (cm)	94 ± 7	114 ± 6	0.0000
Waist (cm)	82 ± 4	111 ± 9	0.0000
Waist-to-height ratio	0.443 ± 0.0	0.607 ± 0.0	0.0000
Total fat mass (kg)	12.7 ± 4.5	39.6 ± 6.4	0.0000
Total fat (% of total body weight)	16.8 ± 5.7	35.5 ± 4.1	0.0000
Android fat mass (kg)	1.4 ± 1.1	4.4 ± 8.3	0.0000
Gynoid fat mass (kg)	3.4 ± 2.8	6.8 ± 1.1	0.0009
Total lean mass (kg)	62.6 ± 6.2	71.6 ± 6.3	0.0040
Metabolic profile			
HbA1c (mmol/mol)	32.3 ± 2.5	31.4 ± 2.4	0.4180
HbA1c (%)	5.1 ± 0.2	5.0 ± 0.2	0.4176
Fasting glucose (mmol/L)	4.7 ± 0.3	4.9 ± 0.4	0.2659
2h-OGTT-glucose (mmol/L)	4.9 ± 1.2	5.3 ± 1.4	0.5200
Fasting insulin (mmol/L)	42 ± 16	94 ± 24 ^a	0.0001
HOMA-IR	1.4 ± 0.5	3.8 ± 1.7 ^a	0.5200
Total cholesterol (mmol/L)	3.7 ± 0.6	4.7 ± 0.8	0.0122
LDL cholesterol (mmol/L)	2.3 ± 0.7	2.9 ± 0.6	0.0468
HDL cholesterol (mmol/L)	1.2 ± 0.2	1.3 ± 0.3	0.5043
Triglycerides (mmol/L)	0.9 ± 0.3	1.7 ± 0.8	0.0225
Total free fatty acids (mmol/L)	0.42 ± 0.15	0.48 ± 0.12	0.3439
Cytokines and hormones			
IL-6 (pg/mL)	0.46 ± 0.22	0.59 ± 0.27	0.2732
TNF-α (pg/mL)	1.44 ± 0.18	1.59 ± 0.32	0.2134
Leptin (ng/mL)	0.72 ± 0.53	8.92 ± 6.60	0.0058
BP, heart rate, and fitness			
Systolic BP (mmHg)	126 ± 10	131 ± 14	0.4396
Diastolic BP (mmHg)	72 ± 7	78 ± 11	0.2176
Resting heart rate (bpm)	57 ± 9	66 ± 8	0.0241
VO _{2peak} (mL/min)	3,779 ± 574	3,989 ± 555	0.4004
VO _{2peak} /FFM (mL/kg/min)	48 ± 6	35 ± 5	0.0000

Data are presented as mean ± SD. OGTT, oral glucose tolerance test; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure; VO_{2peak}, peak oxygen uptake; FFM, fat-free mass.

^an = 8.

2.4-fold in the obese group. The median R_a palmitate per kilogram of fat mass in the obese group was 48%–65% lower than in the lean group (Figure 3B). However, R_a palmitate per

kilogram of fat-free mass was similar in the two groups (Figure 3C). Acute and chronic IL-6 receptor blockade profoundly reduced R_a palmitate in the lean group (Figures 3B and 3C, left panels) during rest, exercise, and recovery (maximal reduction –31%, 95% confidence interval [CI] [–47% to –10%] during exercise on day 1 and –33%, 95% CI [–49% to –13%] at rest on day 21, $p < 0.01$). IL-6 receptor blockade also reduced R_a palmitate during rest, exercise, and recovery in the obese group (maximal reduction –33%, 95% CI [–46% to –16%] during exercise on day 1, $p < 0.01$ and –28%, 95% CI [–42% to –10%] during exercise on day 21, $p < 0.01$), but the effect was significant mainly at time points following chronic IL-6 receptor blockade (Figures 3B and 3C, right panels). Overall, IL-6 receptor blockade appeared to lower R_a palmitate more profoundly in the lean than in the obese group; however, the difference in effect was not significant.

The lower release of free fatty acids in relation to fat mass in the obese group is in line with literature and is considered a physiological adaptation to avoid excessive free fatty acids in the circulation.²⁰ One could therefore speculate that the apparent lesser effect of IL-6 receptor blockade in the obese group is due to the already downregulated free fatty acid release.

IL-6 receptor blockade reduces free fatty acid uptake from the circulation but has no effect on palmitate oxidation

The rate of disappearance of palmitate (R_d palmitate) reflects the uptake of free fatty acids from the circulation. Similar to R_a palmitate, R_d palmitate increased steadily during exercise (median increase by 2.4-fold in the lean group and 2.5-fold in the obese group) and decreased rapidly in recovery. IL-6 receptor blockade lowered R_d palmitate in both groups, with no significant difference in effect between groups (Figure 3D).

Palmitate taken up from the circulation is oxidized or re-esterified. The palmitate oxidation rate (Figure 3E) rose in response to exercise. The median increase in palmitate oxidation rate was 7.7-fold in the lean group and 4.5-fold in the obese group. There was no significant difference in palmitate oxidation rate between groups (saline day). The palmitate oxidation rate in absolute numbers and relative to R_d palmitate was unaffected by IL-6 receptor blockade (Figures 3E and S2). Likewise, whole-body fat oxidation, determined by indirect calorimetry, was unchanged with IL-6 receptor blockade (Table 2). Extracellular palmitate re-esterification, representing the free fatty acids that enter the circulation and are taken up for esterification by tissues, can be derived from R_d palmitate and the palmitate oxidation rate. Acute and chronic IL-6 receptor blockade reduced extracellular palmitate re-esterification during exercise and recovery in the lean group (acutely –1.1 $\mu\text{mol/kg}$ fat-free mass [FFM]/min, 95% CI [–1.9 to –0.3] and chronically –1.1 $\mu\text{mol/kg}$ FFM/min, 95% CI [–1.9 to –0.3], $p < 0.01$ during exercise; acutely –0.9 $\mu\text{mol/kg}$ /min, 95% CI [–1.7 to –0.1] and chronically –0.8 $\mu\text{mol/kg}$ /min, 95% CI [–1.6 to –0.0]), $p < 0.05$ during recovery), whereas it was unaffected by IL-6 receptor blockade at rest in the lean group and throughout the study day in the obese group on day 1 and day 21. This difference in suppression of extracellular palmitate re-esterification between groups was significant during exercise ($p < 0.01$ day 1, $p < 0.05$ day 21). Under

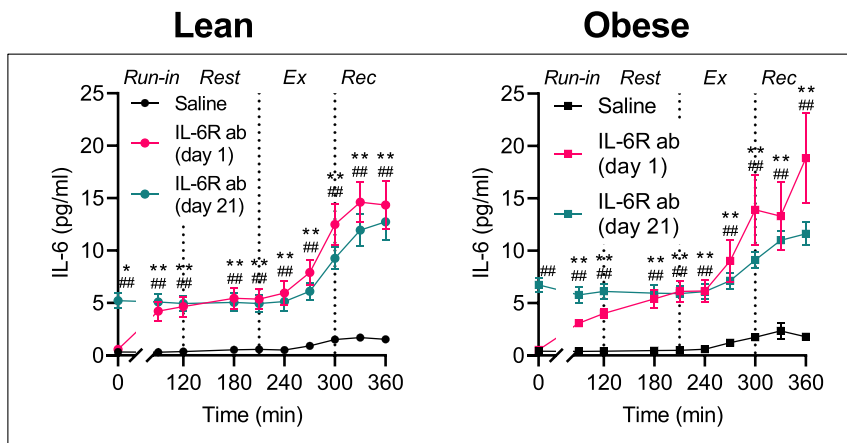


Figure 2. IL-6 receptor blockade increases IL-6 concentration

Plasma concentration of IL-6. Left panel: data from the lean group ($n = 13$). Right panel: data from the obese group ($n = 9$). Data are represented as mean \pm SEM. * $p < 0.05$ day 1 versus saline, ** $p < 0.01$ day 1 versus saline, ## $p < 0.01$ day 21 versus saline using a linear mixed-effects model and Dunnett's method for many-to-one comparisons. IL-6R ab, IL-6 receptor antibody; Ex, exercise; Rec, recovery.

fasting conditions, free fatty acids are mainly re-esterified into TAG in skeletal muscle and the liver (extracellular re-esterification), and TAG can consequently be released from the latter into the circulation. Plasma TAG concentrations were 47%–54% higher in the obese group than in the lean group ($p < 0.05$). TAG concentrations on day 1 were lower than on the saline day already at baseline before IL-6 receptor blockade, and we saw no effect of IL-6 receptor blockade on TAG concentrations (Figure 3F). Thus, it is most likely extracellular fatty acid re-esterification in skeletal muscle that is lowered by IL-6 receptor blockade.

IL-6 receptor blockade had no effect on cholesterol levels in either group (Table S1). This is probably due to the short duration of treatment (21 days).

The findings regarding fatty acid oxidation contrast our hypothesis. Infusion of rhIL-6 in humans has been shown previously to increase whole-body fatty acid oxidation;^{4–6} therefore, we hypothesized that IL-6 receptor blockade reduces palmitate and whole-body fatty acid oxidation. However, that a gain-of-function study cannot be reproduced by a loss-of-function study suggests that different mechanisms are in play. Also, in the rhIL-6 infusion studies, plasma levels of IL-6 ranged from 35–320 pg/mL,^{4–6,21} which is considerably higher than the exercise-induced IL-6 levels in our study. Therefore, it is possible that the exercise-induced IL-6 level in our study was not high enough to stimulate fatty acid oxidation because the increased fatty acid oxidation observed in response to infusion of rhIL-6 was seen with higher concentrations of IL-6.

Previous data regarding extracellular fatty acid re-esterification in humans *in vivo* are limited; nevertheless, infusion of rhIL6 has been shown to increase whole-body extracellular re-esterification.⁶ Thus, the opposite effect (i.e., a reduction in extracellular re-esterification in response to IL-6 receptor blockade), is well in line with this.

IL-6 receptor blockade slightly reduces whole-body lipolysis in obese individuals

As expected, plasma concentrations of glycerol and R_a glycerol, a measure of lipolysis, increased steadily during the exercise bout and decreased rapidly during recovery in the

lean and obese groups (Figures 4A–4C). Similar to R_a palmitate, R_a glycerol per kilogram of fat mass in the obese group was 56%–67% lower than in the lean group (Figure 4B), whereas R_a glycerol per kilogram of FFM was similar between groups (Figure 4C). Glycerol concentrations were unaffected by IL-6 receptor blockade (Figure 4A), and in contrast to our hypothesis, R_a glycerol was also unaffected by IL-6 receptor blockade in the lean group (Figures 4B and 4C, left panels). In the obese group, however, R_a glycerol was reduced significantly in the early recovery phase (–28%, 95% CI [–57% to –10%], $p < 0.01$) and tended to be reduced at rest (–20%, 95% CI [–35% to 0%], $p = 0.055$) and early in the exercise bout (–19%, 95% CI [–35% to 1%], $p = 0.061$) following chronic IL-6 receptor blockade (Figures 4B and 4C, right panels). However, the difference in inhibitory effect of IL-6 receptor blockade on R_a glycerol was not significant between groups.

Given the stimulatory effect of rhIL-6 on lipolysis^{4–6,21} and induction of IL-6 during exercise, we hypothesized that IL-6 receptor blockade would impair exercise-induced lipolysis. However, IL-6 receptor blockade had no acute or chronic effects on exercise-induced lipolysis in lean participants, whereas it inhibited lipolysis during early recovery in obese participants. This inhibitory effect on lipolysis was only present after chronic IL-6 receptor blockade, suggesting that the effect may not be a direct consequence of inhibiting IL-6 but, rather, due to some secondary adaptations (discussed below with effect on hormones). Our data indicate that endogenous IL-6 is not a determinant of resting and exercise-induced lipolysis in lean men but may play a role in regulating lipolysis in obese men. Obesity is often associated with chronically elevated IL-6 levels and is commonly seen as a state of chronic low-grade inflammation.²² IL-6 levels correlate with fat mass⁷ and adipocyte volume²³, and, therefore, elevated IL-6 levels could have a counter-regulatory role in obesity to increase lipolysis.

In our study, the overall impression is that the effect of IL-6 receptor blockade on lipolysis is small. The obese participants were relatively healthy, and IL-6 levels did not differ between the two groups. Thus, one could speculate that the effects of IL-6 receptor blockade would be more pronounced in a metabolically less healthy obese group. It is also possible that blocking the IL-6 signal during a more intense exercise bout to induce higher circulating levels of IL-6, would reveal a greater effect of IL-6 receptor blockade.

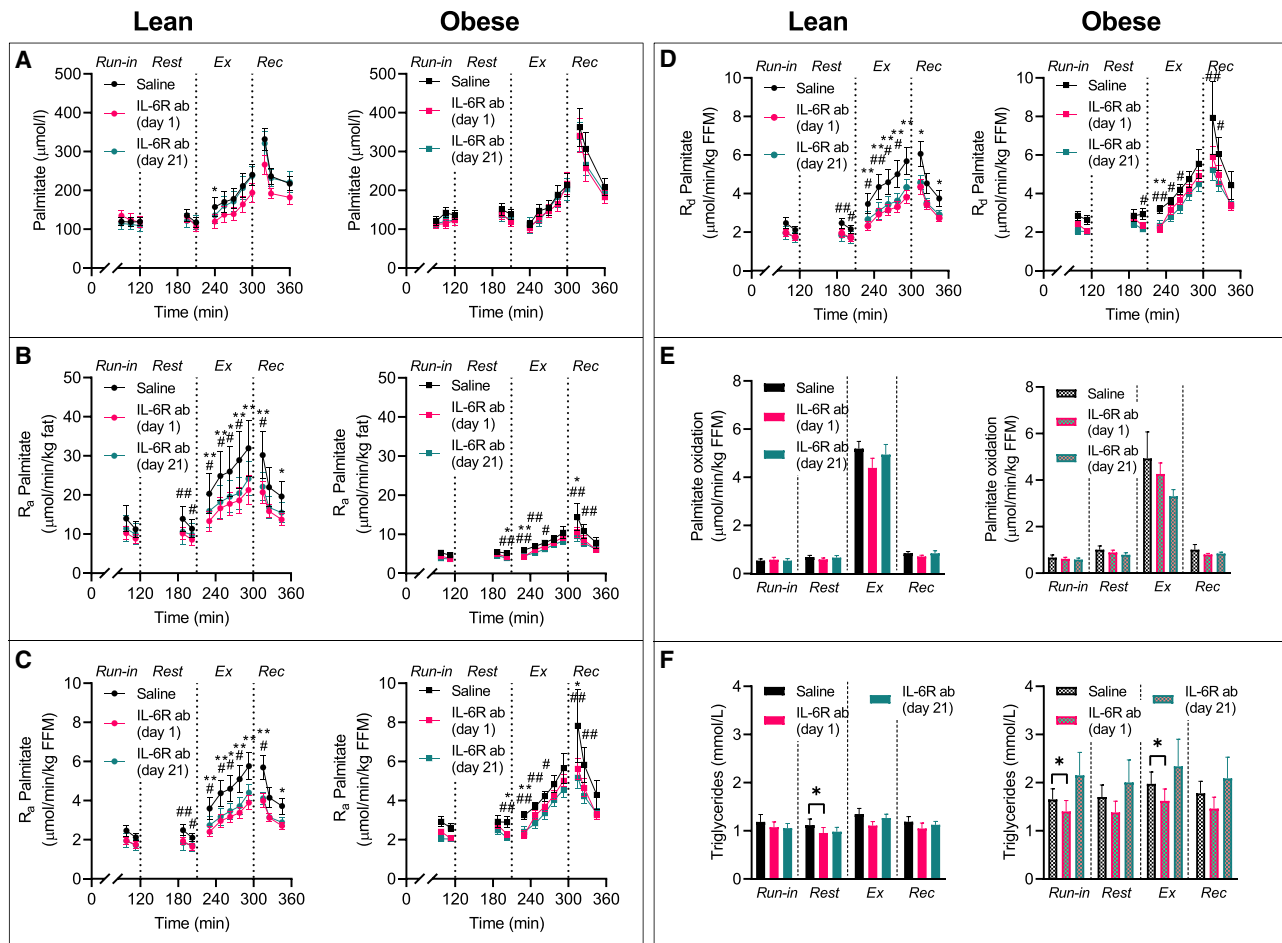


Figure 3. IL-6 receptor blockade reduces release of palmitate into the circulation but has no effect on palmitate oxidation

(A–F) Plasma concentration of palmitate (A), R_a palmitate per kilogram of fat mass (B) and per kilogram of fat free mass (FFM) (C), R_d palmitate per kilogram of FFM (D), palmitate oxidation rate (E), and average plasma concentration of triglycerides in each phase (F). Left panels: data from the lean group ($n = 11$ for palmitate data, $n = 13$ for triglycerides). Right panels: data from the obese group ($n = 9$). Gaps are left at the beginning of each phase because data were calculated based on the average of two consecutive measurements without overlapping phases (see details in the STAR Methods). Data are represented as mean \pm SEM. * $p < 0.05$ day 1 versus saline, ** $p < 0.01$ day 1 versus saline, # $p < 0.05$ day 21 versus saline, ## $p < 0.01$ day 21 versus saline using a linear mixed-effects model and Dunnett’s method for many-to-one comparisons. R_a , rate of appearance; R_d , rate of disappearance. See also Figure S2.

IL-6 receptor blockade reduces free fatty acid mobilization relative to lipolysis

The ratio R_a palmitate to R_a glycerol (Figure 4D) is indicative of the proportion of palmitate re-esterified within the tissue following lipolysis and, therefore, is also indicative of the proportion of palmitate not being released into the circulation following lipolysis.^{24,25} Acute and chronic IL-6 receptor blockade lowered the median ratio in the lean group at rest and during exercise and recovery (maximal reduction -26% , 95% CI $[-40$ to $-9]$, $p < 0.01$ during exercise on day 1), suggesting that IL-6 receptor blockade reduces the efflux of free fatty acids liberated during lipolysis. In the obese group, we saw no significant effect of IL-6 receptor blockade on the R_a palmitate to R_a glycerol ratio. The difference in effect between groups was, however, not significant.

We did not determine the ratio of palmitate to total fatty acids. However, previous studies have reported a 22%–26% contribution of palmitate to total fatty acids.^{26–28} Under these assumptions,

a ratio R_a palmitate to R_a glycerol of 0.4–0.6 would lead to a calculated ratio R_a free fatty acids to R_a glycerol of 1.5–2.7:1, which may seem low compared with the ratio of 3:1 according to textbook knowledge but is in line with our previous studies.^{6,29,30}

Taking the palmitate and glycerol kinetics data together, our main finding is that blocking endogenous IL-6 signaling impairs the efflux of free fatty acids in lean and obese individuals during rest, exercise, and recovery. These data are consistent with previous studies reporting increased whole-body R_a palmitate upon infusion of rhIL-6 in humans.^{5,6,21} However, in those studies, glycerol release was also increased, and, therefore, the change in lipolysis was assumed to underlie the increased R_a palmitate. Intriguingly, we saw no decrease in lipolysis in the lean group, but R_a palmitate was decreased by IL-6 receptor blockade, suggesting that another mechanism than lipolysis is in play. Our data suggest that IL-6 receptor blockade may decrease free fatty acid mobilization by increasing intracellular re-esterification. In

Table 2. IL-6 receptor blockade has no effect on total fat and carbohydrate oxidation

	Lean			Obese		
	Saline	IL-6R ab (day 1)	IL-6R ab (day 21)	Saline	IL-6R ab (day 1)	IL-6R ab (day 21)
RER						
Rest	0.73 ± 0.01	0.75 ± 0.01	0.73 ± 0.01	0.74 ± 0.01	0.74 ± 0.01	0.74 ± 0.01
Exercise	0.85 ± 0.01	0.86 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.86 ± 0.01
Recovery	0.71 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.73 ± 0.01
Oxygen uptake (mL/min)						
Rest	287 ± 5	280 ± 8	298 ± 10	349 ± 11	338 ± 10	348 ± 11
Exercise	2,183 ± 72	2,159 ± 67	2,145 ± 68 ^a	2,066 ± 140	2,025 ± 144	2,036 ± 134
Recovery	308 ± 6	299 ± 7	313 ± 10	372 ± 9	357 ± 12 ^b	365 ± 13
Total fat oxidation (mg/min/kg)						
Rest	1.64 ± 0.09	1.52 ± 0.07	1.71 ± 0.07	1.36 ± 0.07	1.29 ± 0.08	1.31 ± 0.06
Exercise	7.05 ± 0.43	6.56 ± 0.52	6.36 ± 0.47	4.31 ± 0.38	4.22 ± 0.29	4.07 ± 0.43
Recovery	1.98 ± 0.08	1.89 ± 0.08	1.89 ± 0.07	1.56 ± 0.09	1.47 ± 0.07	1.48 ± 0.08
Total carbohydrate oxidation (mg/min/kg)						
Rest	0.54 ± 0.17	0.67 ± 0.17	0.44 ± 0.17	0.49 ± 0.14	0.52 ± 0.14	0.52 ± 0.14
Exercise	17.81 ± 0.79	18.92 ± 0.79	19.00 ± 0.82	12.42 ± 1.60	12.36 ± 1.60	12.81 ± 1.60
Recovery	0.19 ± 0.09	0.27 ± 0.09	0.31 ± 0.09	0.30 ± 0.12	0.32 ± 0.12	0.38 ± 0.12

Data are represented as mean ± SEM.

^ap < 0.01 versus saline day, using a linear mixed-effects model and Dunnett's method for many-to-one comparisons. IL-6R ab, IL-6 receptor antibody; RER, respiratory exchange ratio.

^bp < 0.05 versus saline day using a linear mixed-effects model and Dunnett's method for many-to-one comparisons.

support of this, IL-6 treatment of cultured mouse epididymal adipose tissue has been shown to decrease phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate dehydrogenase kinase 4 (PDK4) mRNA expression, two enzymes essential for glyceroneogenesis and lipogenesis in adipose tissue, and to increase the ratio between released free fatty acids and glycerol, indicating reduced fatty acid re-esterification by IL-6.³¹ Human data regarding the role of endogenous IL-6 in intracellular re-esterification have not been published to date.

Based on our study, it is not possible to come to a conclusion regarding the compartment where IL-6 receptor blockade has its main effect. Given the abovementioned mouse study³¹ and the fact that IL-6 receptor blockade prevents exercise from reducing visceral and cardiac adipose tissue mass,^{12,13} we speculate that the increased intracellular re-esterification mainly takes place in adipose tissue. Adipose tissue of different compartments might be differentially sensitive to IL-6 because of differential IL-6 receptor density.³² Nevertheless, skeletal muscle may contribute to the whole-body effect; as mentioned above, the reduced extracellular re-esterification of fatty acid from the circulation likely takes place in skeletal muscle. Therefore, one could speculate that if the release of free fatty acids from lipolysis of intramyocellular TAG is reduced, then these free fatty acids could be re-esterified directly or enter oxidation in the myocyte, which, in turn, would lead to less fatty acids taken up from the circulation to be oxidized.

IL-6 receptor blockade has no effect on glucose kinetics

During prolonged exercise, increased glucose demand by skeletal muscle is paralleled by increased endogenous glucose produc-

tion (mainly hepatic glycogenolysis and gluconeogenesis); thus, plasma glucose levels can still be constant after 2 h of exercise in well-nourished individuals.³³ In the present study, plasma glucose levels decreased slightly during the exercise bout in the lean group and remained unchanged in the obese group (Figure 4E). In both groups, endogenous glucose production (given by R_a glucose) and glucose disposal (given by R_d glucose) increased continuously during the exercise bout and decreased rapidly in recovery (Figures 4F and 4G). Mean R_a glucose increased 2.8-fold in the lean group and 2.4-fold in the obese group, whereas mean R_d glucose increased 2.9-fold in the lean group and 2.4-fold in the obese group. Glucose levels, R_a glucose, and R_d glucose were unaffected by IL-6 receptor blockade in both groups, as was whole-body carbohydrate oxidation (Table 2).

That glucose kinetics were unaffected by IL-6 receptor blockade is in line with previous studies showing that infusion of rhIL-6 into resting humans had no effect on R_a glucose, R_d glucose, or plasma glucose levels.^{6,34} However, because infusion of rhIL-6 into exercising humans increased endogenous glucose production,³⁵ it could be expected that IL-6 receptor blockade would have the opposite effect.

Our results suggest that endogenous IL-6 plays no major role in regulating whole-body glucose kinetics and metabolism at rest, during moderate-intensity exercise, and in the recovery phase in lean and obese men.

IL-6 receptor blockade reduces cortisol and glucagon levels

Cortisol levels declined continuously during the 3.5-h run-in and resting phase, likely because of the circadian rhythm of this

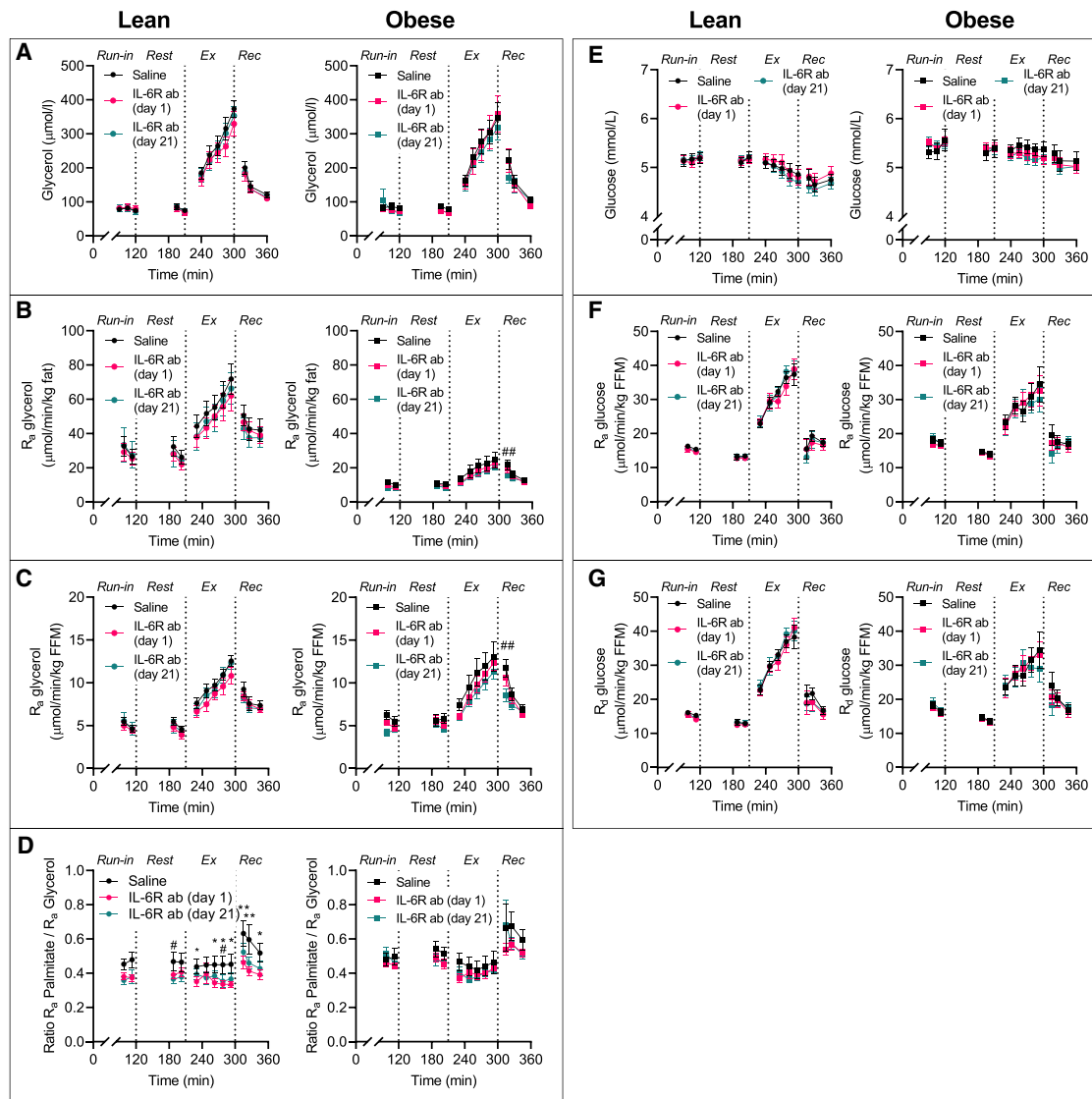


Figure 4. IL-6 receptor blockade reduces lipolysis in obese individuals and has no effect on glucose kinetics

(A–G) Plasma concentration of glycerol (A), R_a glycerol per kilogram of fat mass (B) and per kilogram of FFM (C), ratio of R_a palmitate to R_a glycerol (D), plasma glucose concentration (E), R_a glucose (F), and R_d glucose (G). Left panels: data from the lean group ($n = 12$). Right panels: data from the obese group ($n = 9$). Gaps are left at the beginning of each phase because data were calculated based on the average of two consecutive measurements without overlapping phases (see details in the STAR Methods). Data are represented as mean \pm SEM. * $p < 0.05$ day 1 versus saline, ** $p < 0.01$ day 1 versus saline, # $p < 0.05$ day 21 versus saline, ## $p < 0.01$ day 21 versus saline using a linear mixed-effects model and Dunnett’s method for many-to-one comparisons.

hormone. Cortisol levels rose in response to the exercise bout and dropped again during recovery (Figure 5A). Chronic IL-6 receptor blockade reduced cortisol levels in both groups. The reduction was significant only during rest and recovery, and the absolute change was more pronounced in the obese than in the lean group during recovery ($p < 0.05$ at 330 min).

Infusion of high doses of rhIL-6 into humans increases adrenocorticotrophic hormone and cortisol levels.^{36,37} To our knowledge, reduced cortisol levels after chronic IL-6 receptor blockade have not been reported previously. It is possible that the decrease in cortisol levels by IL-6 receptor blockade in the obese group contributed to the observed reduction in lipolytic rate. However,

the literature is not conclusive regarding the lipolytic role of cortisol. Cortisol-deficient individuals with adrenal insufficiency tend to have increased lipolysis,³⁸ and administration of exogenous cortisol has been shown to stimulate,^{39,40} inhibit,⁴¹ or have no effect on lipolysis^{42,43} in healthy and obese humans.

Insulin concentrations were higher in the obese group than in the lean group throughout the saline day. Insulin levels decreased steadily during exercise and increased steeply immediately after the end of exercise (Figure 5B). IL-6 receptor blockade had no relevant effect on insulin concentrations.

As expected, exercise induced a rise in glucagon levels in both groups. IL-6 receptor blockade had no effect on glucagon levels

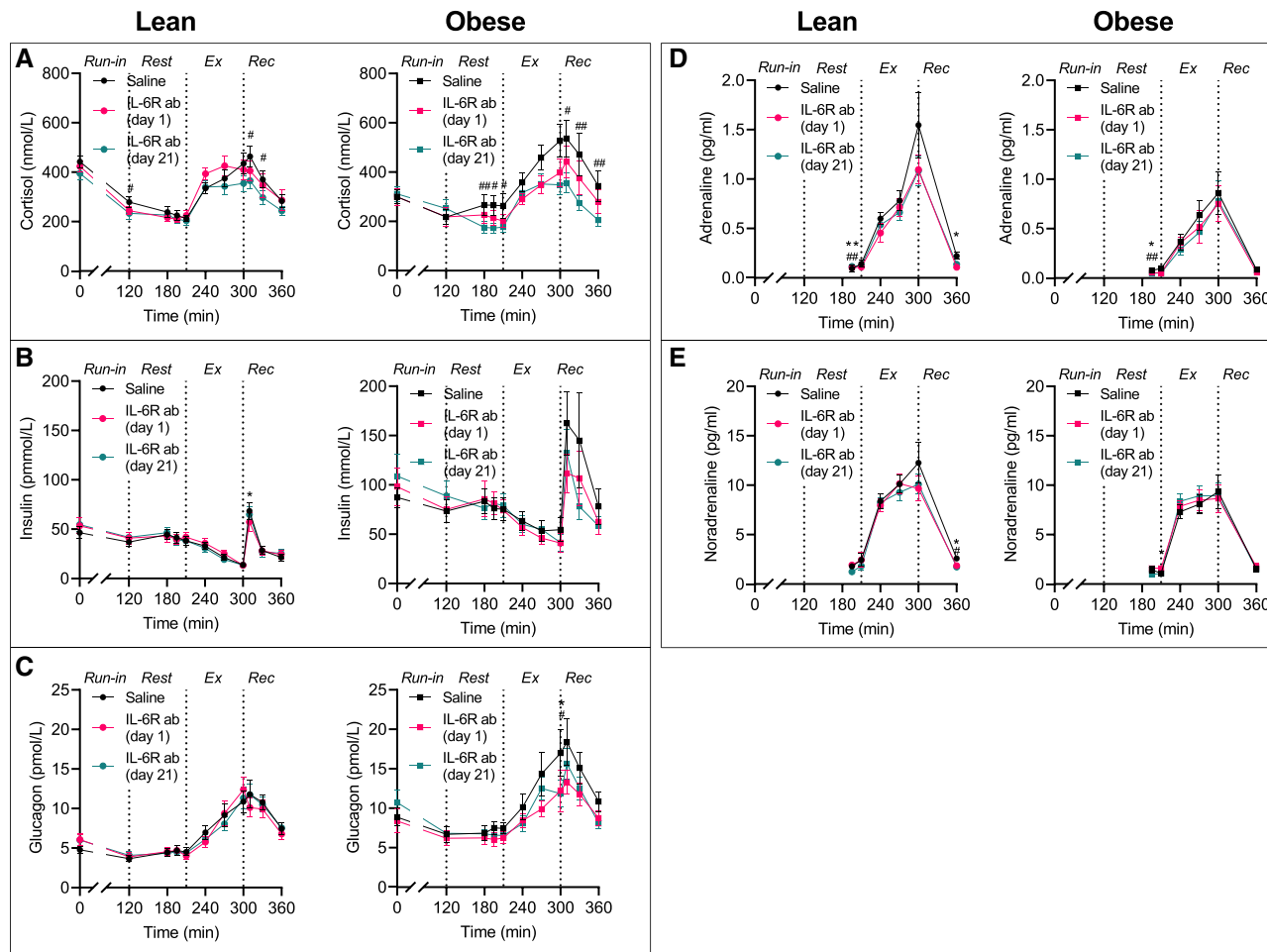


Figure 5. IL-6 receptor blockade decreases cortisol and glucagon levels

(A–E) Plasma concentrations of cortisol (A), insulin (B), glucagon (C), adrenaline (D), and noradrenaline (E). Left panels: data from the lean group ($n = 13$). Right panels: data from the obese group ($n = 9$). Data are represented as mean \pm SEM. * $p < 0.05$ day 1 versus saline, ** $p < 0.01$ day 1 versus saline, # $p < 0.05$ day 21 versus saline, ## $p < 0.01$ day 21 versus saline using a linear mixed-effects model and Dunnett’s method for many-to-one comparisons.

in the lean group but reduced exercise-induced levels in the obese group on day 1 and day 21 of IL-6 receptor blockade (Figure 5C), and this reduction was significantly different from the lean group (day 1 $p < 0.01$, day 21 $p = 0.08$).

Reduced exercise-induced glucagon levels under chronic IL-6 receptor blockade have not been reported previously. Nevertheless, IL-6 has been shown previously to stimulate glucagon secretion,⁴⁴ and administration of rhIL-6 into healthy humans also increased glucagon levels.^{21,45,46} Our data support the theory that IL-6 may play a physiological role in regulating glucagon in the context of exercise and that this might only become relevant in the obese state. Therefore, it is also possible that the decrease in glucagon levels by IL-6 receptor blockade in the obese group contributed to the reduced lipolytic rate.

Circulating catecholamine levels increased in response to exercise and decreased again during recovery (Figures 5D and 5E). No major effect of IL-6 receptor blockade on circulating cate-

cholamine levels were observed. However, this does not allow us to reach a conclusion regarding the sympathetic activity at tissue level.

DISCUSSION

Here we show that blocking endogenous IL-6 signaling changes the turnover of fat, promoting its storage rather than mobilization. This effect was observed in lean and obese men. Mobilization of free fatty acids was impaired by IL-6 receptor blockade in both groups. However, in contrast to the lean group, IL-6 receptor blockade additionally slightly impaired lipolysis in the obese group, thus affecting two parts of the fat mobilization pathway. The fact that these effects were observed in response to acute as well as chronic IL-6 receptor blockade suggests a direct effect of IL-6. Nonetheless, indirect long-term effects of IL-6 receptor blockade may play a role as well because cortisol and glucagon levels were also lowered.

It is intriguing that circulating concentrations of IL-6, a multi-functional molecule increased in response to exercise and infection, plays such a key role in mobilizing fat from its stores and, thus, energy availability. The exercise-induced IL-6 response has been shown to depend on the presence of glucose and glycogen. Ingestion of glucose lowers exercise-induced IL-6,^{47–49} and low muscle glycogen content increases exercise-induced IL-6;^{50,51} thus, it appears that one role of IL-6 may be to mobilize fat as an energy source at times of low glucose availability.³ Such a role of IL-6 may extend to conditions of infection where activated immune cells secrete IL-6. On a speculative note, it is possible that immune-cell-derived IL-6 serves to ensure sufficient glucose for the immune cells to mount a robust immune response by stimulating mobilization of fat to be used as energy by other organs.

Our findings support a catabolic function of IL-6 in fat metabolism and show that blocking endogenous IL-6 signaling promotes storage of fat, which, in the long run, may lead to expansion of whole-body fat mass but also accumulation of intramyocellular fat. Our data have clinical implications; individuals with rheumatoid arthritis are treated with tocilizumab,¹⁴ and IL-6 antagonism is about to enter phase 3 for treatment of cardiovascular disease.¹⁵ Thus, understanding the effects of anti-IL-6 therapy on metabolism is of great importance for these individuals.

Limitations of the study

The present study has some limitations. First, this study was not randomized; therefore, an effect of habituation cannot be excluded. Second, we assumed that all glycerol appears in the blood after lipolysis. However, glycerol released from muscle lipolysis might be directly oxidized instead of released into the circulation and therefore lead to underestimation of the rate of lipolysis.⁵² Furthermore, considering the greater variability within the obese group, a higher number of participants may have given more robust results regarding the small decrease in lipolysis by IL-6 receptor blockade. Nevertheless, the effect size is minor, and overall, IL-6 does not appear to play a major role in regulating glycerol appearance. Third, our calculated ratio of *Ra* free fatty acids to *Ra* glycerol of 1.5–2.7:1 may seem low compared with the ratio of 3:1 according to textbook knowledge. However, these numbers are in line with our previous studies,^{6,29,30} thus, our data are robust enough to support our conclusions. Fourth, our whole-body model cannot reveal any mechanistic insights; therefore, further experiments, such as adipocyte and myocyte experiments examining the different pathways of lipid metabolism and measurements of arteriovenous differences across tissues along with tissue biopsies, are required to clarify how IL-6 receptor blockade regulates re-esterification on a molecular level and whether the effect is global or tissue specific. Finally, all participants were male; therefore, our findings might not translate to a female population.

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2021.100396>.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.E.; methodology, H.E., G.v.H., P.P., and K.K.; formal analysis, B.T. and C.S.; investigation, B.T., M.P., and H.E.; resources, B.K.P. and G.v.H.; writing – original draft, B.T. and H.E.; writing – review & editing, all authors; visualization, B.T. and H.E.; funding acquisition, H.E., B.K.P., and B.T.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

RESOURCE AVAILABILITY

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Tocilizumab. Recombinant humanized monoclonal IL-6 receptor antibody (20 mg/mL)	Roche Pharma AG	RRID: AB_2459656
Critical commercial assays		
Glucagon	Mercodia, Uppsala, Sweden	10-1286-01
Epinephrine, norepinephrine	LDN Immunoassays and Services, Nordhorn, Germany	BA E-6500
IL-6, TNF- α (V-PLEX)	Meso Scale Diagnostics, Rockville, Maryland, US	K151A9H-2
IL-6 (S-PLEX)	Meso Scale Diagnostics, Rockville, Maryland, US	K151B3S-1
Leptin (V-PLEX)	Meso Scale Diagnostics, Rockville, Maryland, US	K151V5D
Software		
R studio, version 3.6.2, using the “lme4” and “emmeans” packages		

Lead contact

Helga Ellingsgaard (helga.ellingsgaard@regionh.dk)

Materials availability

This study did not generate new unique reagents.

Data and code availability

Data and code supporting this study have not been uploaded to a public repository but are available from the corresponding author on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Participants and screening

Between July 1 2019 and April 3 2020, 15 lean and 11 obese individuals were enrolled at the Centre for Physical Activity Research (CFAS) in the Capital Region of Denmark, 13 lean and 9 obese participants completed the study.

Inclusion criteria were male gender, age ≥ 18 years, a normal BMI (≥ 18.5 and ≤ 25 kg/m²) for the lean group and a BMI ≥ 30 and ≤ 40 kg/m² for the obese group. Exclusion criteria were the use of any medication, smoking, evidence of severe thyroid, heart, lung, liver, kidney or inflammatory disease, current infection and (pre-)diabetes. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg Communities, Denmark, reported to the Danish Data Protection (P-2019-166), registered at [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT03967691) and performed according to the Declaration of Helsinki. All participants gave written informed consent.

Study design

This was a placebo-controlled, non-randomized, single-blinded, crossover study comprising one screening visit and three study visits. Due to the long elimination half-life of tocilizumab, the order of study visits was not randomized and therefore, only the participants were blinded. The first and second study visits were scheduled seven days apart, the second and third visits 21 days apart.

On the screening day, an electrocardiogram (ECG), measurements of blood pressure, height, weight, hip, waist, body composition (DXA scan) and cardiorespiratory fitness (VO_{2max} test), and an oral glucose tolerance test (OGTT) along with a baseline blood sample were performed.

On study days, participants presented fasted (≥ 10 h, no alcohol or caffeine) at 8:00 at CFAS after refraining from exercise 48h prior to each study day. Participants voided, got their body weight measured and were placed in bed. The experimental room temperature was kept constant (20–24°C, mean difference between study visits within-individual 0.8°C). Participants were only permitted to drink water *ad libitum* during the experiments. One antecubital venous catheter was placed in each arm, one for infusion of stable isotopes and tocilizumab or placebo (0.9% saline), and the other for blood sampling. A primed, continuous infusion of stable isotopes was started at time point 0 min. Tocilizumab (second study visit) or normal saline serving as placebo (first and third study visit) were infused over the first 60 minutes. Each study day consisted of four phases: a run-in phase during which isotopic equilibrium was achieved, a 90 min resting phase, a 90 min exercise bout and a 60 min recovery phase. Indirect calorimetry measurements were obtained during the last 30 min of each phase followed by an expired breath sample.

METHOD DETAILS

Anthropometric and blood pressure measurements

Body weight was measured with 0.1 kg accuracy (ADP calibrated electronic scale), height was measured to the nearest 0.1 cm (Holtain stadiometer, Holtain Ltd, Crymych, UK), waist and hip circumference were measured to the nearest 0.1 cm. Resting blood pressure and heart rate were measured with an automated oscillometric device on the upper left arm (Omron 705IT, Omron Corporation, Kyoto, Japan).

Body composition

Body composition was determined by dual X-ray absorptiometry (Lunar Prodigy; GE Medical Systems, Madison, WI, USA). Total lean body mass (not including bone mass) and total fat mass were used to normalize substrate kinetics data to fat free mass and fat mass, respectively.

Glucose tolerance

An oral glucose tolerance test was performed with 75 g glucose. Plasma glucose was measured at 0 min, 60 min and 120 min, insulin was measured at 0 min.

Cardiorespiratory fitness

Peak power output (Watt_{max}) and peak oxygen uptake ($\text{VO}_{2\text{peak}}$) were measured using an incremental exercise test on an electronically braked bicycle ergometer (Monark Exercise AB, Vansbro, Sweden) with concurrent breath-by-breath measurement of VO_2 using an indirect calorimetry system (Quark CPET, Cosmed, Rome, Italy). After a 5 min warm-up at 100 W, the workload was increased by 25 W every minute until exhaustion. Watt_{max} was calculated as $\text{Watt}_{\text{max}} = W_{\text{completed}} + 25 * (60/t)$ where $W_{\text{completed}}$ is the last fully completed workload and t is time (s) at the workload during which exhaustion occurred. The test was valid if the respiratory exchange ratio was > 1.1 , a plateau in oxygen uptake was reached or the heart rate had reached the estimated maximal heart rate ± 10 beats⁵³ and had otherwise to be repeated. Heart rate was continuously monitored with a Garmin heart rate belt (Garmin HRM-Dual, Garmin, USA).

Diet and physical activity

Participants were asked to avoid food naturally enriched in ^{13}C and to keep their diet similar for 2 days before each study day by use of self-reported dietary records and to maintain their free-living physical activity and eating habits during the study.

Tocilizumab and placebo

The recombinant humanized monoclonal IL-6 receptor antibody tocilizumab (Roche, Basel, Switzerland) was reconstituted in 100 mL saline 0.9% and infused over 60 min at a dose of 8 mg/kg body weight or a maximum of 800 mg (day 1). 100 mL of 0.9% saline served as placebo (saline day and day 21).

Indirect calorimetry

VO_2 and VCO_2 were measured with indirect calorimetry (Quark B2, Cosmed, Italy) at time points 90 min, 180 min, 270 min and 330 min for 30 minutes each. A ventilated hood was used while participants were in bed, a face mask was used during the exercise bout. Measurements from the last 10 min of each calorimetry at rest and in recovery, and the last 30 min during exercise were averaged and used for further analysis.

Exercise bout

A 90-minute exercise bout was performed on an electronically braked bicycle ergometer (Monark Exercise AB, Vansbro, Sweden) with the goal of maintaining a rating of perceived exertion between 13 and 15 using the Borg 6–20 scale. On the first study visit, the initial workload was set at 40% of Watt_{max} as determined in the incremental exercise test. Hereafter, the workload was adjusted every 15 minutes to ensure that the perceived exertion was within the target range and the workload was recorded throughout the exercise bout. On study visit two and three, an identical exercise bout was performed, i.e., the workload was continuously adjusted so the

workload always matched the workload used on study visit one irrespective of the perceived exertion on study visit two and three.

Stable isotopes and substrate kinetics

All stable isotopes were purchased from Cambridge Isotope Laboratories (Andover, MA). After obtaining baseline blood and expired breath samples for the measurement of background enrichment, a priming bolus of Na[H¹³CO₃] (1.5 μmol/kg), a primed constant infusion of [6,6-D₂]glucose (0.4 μmol/kg/min, prime 17.6 μmol/kg), a primed constant infusion of [1,1,2,3,3-D₅]glycerol (0.1 μmol/kg/min, prime 1.5 μmol/kg) and a constant infusion of K-[U-¹³C₁₆]palmitate (0.015 μmol/kg/min) were started and maintained until time point 360 min.

Calculations

For each participant and study day, the actual isotope infusion rate was calculated from the infusate concentration multiplied by the infusion flow rate.

Whole body measurements of the rate of appearance (R_a) and disappearance (R_d) of glucose, glycerol and palmitate were calculated using the non-steady-state equations of Steele⁵⁴ adapted for stable isotopes.⁵⁵

$$R_a = \frac{F - pV \left(\frac{C_2 + C_1}{2} \right) \left(\frac{E_2 - E_1}{t_2 - t_1} \right)}{\frac{E_2 + E_1}{2}}$$

$$R_d = R_a - pV \left(\frac{C_2 - C_1}{t_2 - t_1} \right)$$

where F is the isotopic infusion rate (μmol/min), E_1 and E_2 are the blood isotope enrichment of glucose, glycerol or palmitate (TTR) at time 1 (t_1) and 2 (t_2) (min) respectively, C_1 and C_2 are the plasma concentrations at t_1 and t_2 (mmol/l for glucose and μmol/l for glycerol and palmitate) respectively. pV is the volume of distribution, 0.07 and 0.18 (kg body weight)⁻¹ for glucose during rest and exercise respectively, 0.04 and 0.13 (kg body weight)⁻¹ for palmitate and glycerol respectively.

Whole body carbohydrate oxidation was calculated using stoichiometric equations.^{56,57} Total carbohydrate oxidation was determined by converting the rate of carbohydrate oxidation to its molecular equivalent with the molecular weight of glucose being 180 g/mol:

$$\text{Carbohydrate oxidation} = \frac{4.585 V_{CO_2} - 3.226 V_{O_2}}{180}$$

Due to the variation of V_{CO_2} and V_{O_2} measurements carbohydrate oxidation based on above equation may sometimes take a negative value when energy production is maximally relying on fatty acid oxidation. A negative value has no physiological meaning and is therefore considered equal to 0 (45 out of 195 measurements).

Whole body fatty acid oxidation was calculated using stoichiometric equations.^{56,57} Total fatty acid oxidation was determined by converting the rate of TAG oxidation to its molecular equivalent with the assumption that the average molecular weight of TAG is 860 g/mol and multiplied by three to express fatty acid oxidation in fatty acid units because each TAG molecule hydrolyzed yields three fatty acid molecules:

$$\text{Fatty acid oxidation} = \frac{1.695 V_{O_2} - 1.701 V_{CO_2}}{860} \times 3$$

Palmitate oxidation rates were calculated as follows:

$$\text{Palmitate oxidation rate} = \frac{E_{CO_2} \times V_{CO_2}}{E \times a_r}$$

$$\% R_d \text{ palmitate oxidized} = \frac{\text{palmitate oxidation rate}}{R_d} \times 100\%$$

where E_{CO_2} is the breath ¹³C/¹²C ratio, V_{CO_2} is the carbon dioxide output (μmol/min), E is the blood enrichment of palmitate (TTR) and a_r is the acetate correction factor as determined in previous studies.^{30,58,59} As we used an a_r based on previous studies, this may lead to an over- or underestimation in absolute values, the extent of which would, however, be constant between study visits and intra-individually. Therefore, it is unlikely to affect our statistical analyses on the effect of IL-6 receptor blockade.

Assuming that 1) glycerol appears in the circulation only as a product of lipolysis, 2) glycerol cannot be directly incorporated into TAG and that 3) TAG undergoes complete hydrolysis, extracellular palmitate re-esterification was calculated as follows:

$$\text{Extracellular palmitate re-esterification} = R_d \text{ palmitate} - \text{Palmitate oxidation rate}$$

Since the calculation of palmitate oxidation as well as extracellular re-esterification rates also rely on VCO_2 measurements, these results should be interpreted with caution.

Blood samples and analyses

At each study visit, blood samples for the calculation of substrate kinetics were obtained at time points 0, 90, 105, 120, 180, 195, 210, 220, 240, 255, 270, 285, 300, 310, 320, 330 and 360 min. Stable isotopically-labeled tracers in plasma and breath samples were quantified at the Clinical Metabolomics Core Facility at Rigshospitalet as previously described.^{30,60} Insulin, cortisol, free fatty acids and TAG were measured at time points 0, 120, 180, 195, 210, 240, 270, 300, 310, 330 and 360 min at the Department of Clinical Biochemistry at Rigshospitalet. Insulin, c-peptide and cortisol were analyzed using the COBAS 8000 e801 immunoassay system (Roche Diagnostics GmbH), TAG with the COBAS c702 photometric assay system and free fatty acids with the COBAS 8000 c502 photometric assay system. Glucagon was measured at time points 0, 90, 105, 120, 180, 195, 210, 220, 240, 255, 270, 285, 300, 310, 320, 330 and 360 min by ELISA (Mercodia, Uppsala, Sweden); catecholamines were measured at time points 195, 210, 240, 270, 300 and 360 min by ELISA (LDN Immunoassays and Services, Nordhorn, Germany); IL-6 was measured at time point 0 min on the first study visit with an ultra-sensitive electrochemiluminescence assay (S-PLEX, Meso Scale Diagnostics, Rockville, Maryland, US) and together with leptin at time points 0, 90, 120, 180, 210, 240, 270, 300, 330, 360 min on all study visits by multi-array electrochemiluminescence (V-PLEX, Meso Scale Diagnostics, Rockville, Maryland, US).

Blood samples for plasma were immediately centrifuged (3500 rpm, 10 min, 4°C) and left on dry ice until stored at -80°C . Blood samples for serum were left at room temperature for 30 min, centrifuged (3500 rpm, 10 min, 4°C) and left on dry ice until stored at -80°C .

QUANTIFICATION AND STATISTICAL ANALYSIS

Sample size

No study has previously investigated substrate metabolism in the presence of IL-6 receptor blockade, therefore, the sample size of this explorative study was determined based on previous studies where stable isotopes have been used to study substrate kinetics.^{5,61–63}

Statistical analysis

Data were analyzed in R studio, version 3.6.2, using the “lme4” and “emmeans” packages. Descriptive statistics are represented as mean \pm SD. We applied repeated-measures linear mixed-effects models, using time point, study visit, time point \times study visit interaction as categorical fixed effects and individual participants as a random effect, followed by post hoc Dunnett’s many-to-one comparisons adjustment to determine differences between day 1 (acute tocilizumab) and day 21 (chronic tocilizumab) to saline day at each time point and each time point to resting values on the same study visit. Normal distribution of the residuals and homogeneity of variances were checked to fulfill the assumptions for using the linear models. Variables not meeting the model assumptions were logarithmically transformed for optimal model fit. A p -value < 0.05 was considered significant. A secondary mixed-effects model using time point, group, day and time point \times group \times day interaction was used to explore differences in effect of acute and chronic IL-6 receptor blockade between the lean and obese groups. For all outcomes plotted in graphs, the raw mean and SEM are shown for each time point. Estimated same-visit and between-visit changes with 95%-CI were extracted from the mixed-effect model. For log-transformed variables, differences were analyzed on a log-scale and reported as back-transformed relative difference with 95% confidence intervals. For substrate kinetics data, statistical analyses were done for time point 195 min onward. In the lean group, statistical analysis was done with $n = 12$ for glycerol and glucose kinetics and $n = 11$ for palmitate kinetics due to sample measurement problems, the remaining analyses were done with $n = 13$. In the obese group, all analyses were done with $n = 9$.