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The Acute Cytokine Response to 30-Minute Exercise Bouts Before and After 8-Week Endurance Training in Individuals With Obesity

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Abstract

Context: One acute bout of exercise leads to a rapid increase in the systemic cytokine concentration. Regular exercise might alter the cytokine response, in particular in beforehand untrained and obese individuals.

Objective: Using a proximity extension assay, we studied the effects of acute exercise as well as endurance training on a panel of 92 cytokines related to inflammation.

Methods: A total of 22 individuals (30 ± 9 years; peak oxygen uptake [VO_2peak] 25.2 ± 4.2 mL/[kg × min]; body mass index [BMI] 31.7 ± 4.4) participated in an 8-week endurance exercise intervention. Blood samples were collected before and immediately after 30 minutes' ergometer exercise at 80% VO_2peak.

Results: Before and after the training intervention, 40 and 37 cytokines, respectively, were acutely increased more than 1.2-fold (Benjamini-Hochberg [BH]-adjusted $P < .05$). The exercise intervention did not change the acute increase in cytokines nor the resting cytokine levels, whereas fitness was improved and adiposity reduced. The increase in fitness led to a slight increase in power output when exercising at the same heart rate, which might explain the comparable increase in cytokines before and after the intervention. The largest acute increase was found for OSM, TGFA, CXCL1 and 5, and TNFSF14 (≥ 1.9-fold, BH-adjusted $P < .001$). The transcript levels of these proteins in whole blood were also elevated, particularly in the trained state. Only the acute increase in IL6 (1.3-fold) was related to the increase in lactate, confirming the lactate-driven secretion of IL6.

Conclusion: Our comprehensive proteomics approach detected several underexplored serum exerkines with up to now less understood function in the adaptation to exercise.

Key Words: cytokines, acute exercise, proximity extension assay, IL6, OSM, lactate

Abbreviations: BH, Benjamini-Hochberg; BMI, body mass index; cDNA, complementary DNA; CXCL, C-X-C motif chemokine ligand; ELISA, enzyme-linked immunosorbent assay; HbA_{1c}, glycated hemoglobin A_{1c}; IAT, individual anaerobic threshold; IL6, interleukin 6; ISI_{Mats}, insulin sensitivity index estimated by Matsuda method; MCP, monocyte chemoattractant protein; NK, natural killer; OGTT, oral glucose tolerance test; OSM, oncostatin M; qPCR, quantitative polymerase chain reaction; TGFA, transforming growth factor α; TNF, tumor necrosis factor; TNFSF, tumor necrosis factor superfamily member; VO_2max, maximum oxygen uptake; VO_2peak, peak oxygen uptake.

The acute cytokine response to one single bout of exercise was first observed in 1983 (1) in the form of pyrogenic activity of plasma obtained from humans after exercise. Later on, this effect on body temperature was attributed to the increase in

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plasma interleukin-6 (IL6), which was found to be released from the exercising leg (2). This kicked off not only 1 but 2 emerging research fields. The first is the immunomodulatory function of exercise and its relevance in the prevention of chronic metabolic diseases (3) and of aging-associated disorders (4). The second is the field of myokines and other exercise-induced secreted factors that support the functional adaptation of the organism to repeated bouts of exercise and contribute to the beneficial effect of regular physical activity on human health (5). The enormous interest in this research area led to the discovery that several cytokines are elevated in the blood stream after one acute bout of exercise including the interleukins IL1β, IL4, IL13, IL8, IL10, and IL15, interleukin receptor antagonist IL1Ra, colony-stimulating factors, tumor necrosis factor (TNF), and chemokine ligands, such as CCL2 and CX3CL1 (6–8). The acute release of cytokines with mainly anti-inflammatory properties, such as IL10, IL1Ra, and IL6, is considered one of the potent mechanisms by which exercise modulates the function of monocytes and macrophages, directing them toward a less proinflammatory phenotype (6). Notably, many cells are capable of producing cytokines. The systemic concentration always reflects the sum of the release from all sources minus the clearance rate. Measuring the arteriovenous difference over the exercising leg validated the release of some, but not all, cytokines showing increased systemic blood levels from skeletal muscle (5). Other sources for cytokine release into the circulation in response to exercise are natural killer (NK) cells (9), monocytes, macrophages, and T cells (10).

Most previous studies have focused on small, predefined sets of cytokines rather than evaluating the broad cytokine response in an unbiased approach. Moreover, low physical fitness as well as an increased amount of visceral and subcutaneous fat, which harbors a relevant number of macrophages and T cells, might influence the cytokine pattern after acute exercise. Fitness and adiposity both are improved by endurance training, which might change the acute cytokine response. To address this question we made use of the recently developed proximity extension analysis, which allows the simultaneous measurement of a large number of inflammatory cytokines (11). We applied this technique to blood samples collected before and 5 minutes after one 30-minute ergometer exercise bout at 80% peak oxygen uptake (VO_2peak). The participants were middle-aged, untrained, and had overweight or obesity (12). After the first exercise bout, they underwent an 8-week endurance exercise training with 3 60-minute sessions per week followed by the second 30-minute ergometer exercise bout with collection of blood samples. We investigated the acute regulation of a cytokine panel covering 92 analytes and assessed whether this response is altered after the 8-week training period. Furthermore, we analyzed the influence of fitness and metabolic parameters on the cytokine response.

Material and Methods

Study Participants and Design

The participants and design of the study were described recently (12) (NCT03151590 at Clinicaltrials.gov). Healthy individuals with less than 120 minutes of physical activity per week and a body mass index (BMI) greater than 27 were recruited. They underwent incremental cardiopulmonary exercise testing before and after the intervention. The individual anaerobic threshold (IAT) was calculated as the lactate concentration of 1.0 mmol/L above the lowest value of the lactate to performance ratio (13). VO_2peak was defined as the mean VO_2 over the last 20 seconds before cessation of exercise and assessed by metabolic gas analysis (MetaLyzer 3B and MetaMax 3B, Cortex Biophysics GmbH). The day after the fitness test, the participants performed 30 minutes of bicycle ergometer exercise at their individual training intensity set to 80% of VO_2peak. Forty-five minutes before this acute exercise visit, the participants had a standardized breakfast (1 bun, 20 g butter, 1 slice of cheese, 150 g apple puree, water ad libitum). Blood samples were collected in the fasted state and 5 minutes after the exercise bout. After the first acute exercise day, the participants performed an 8-week supervised aerobic endurance training at 80% VO_2peak (3 sessions per week consisting of 30 minutes' treadmill walking and 30 minutes' bicycle ergometer exercise). The intervention was concluded by a second fitness test and a second 30-minute ergometer exercise that were performed on 2 successive days. Exercise intensity was adjusted to and controlled by the heart rate corresponding to 80% VO_2peak. The heart rate was kept constant throughout the intervention, resulting in an increase in exercise intensity during the intervention in most participants, as detailed later. From 22 participants, samples of the 4 time points of the 2 acute exercise bouts were available and used for the analysis of cytokines. All participants gave written informed consent, and the study protocols were approved by the ethics committee of the University of Tübingen and in accordance with the Declaration of Helsinki.

Clinical Analyses and Anthropometry

Glucose and lactate were measured with an ADVIA XPT clinical chemistry analyzer and insulin with an ADVIA Centaur XPT immunoassay system (both Siemens Healthineers). Glycated hemoglobin A_{1c} (HbA_{1c}) was measured by HPLC (Tosoh Bioscience). Peripheral blood cell counts were performed using an XN-10 hematology analyzer (Sysmex). Body fat mass and distribution were measured by magnetic resonance imaging (14). Subcutaneous and visceral adipose tissue was measured from femoral head to humeri. Insulin sensitivity was assessed after an overnight fast by 2 75-g oral glucose tolerance tests (OGTTs), performed pre intervention and 5 days after the last exercise bout, respectively. The insulin sensitivity index was estimated by the method of Matsuda and DeFronzo (ISI_{Mats}) (15). Blood samples for clinical routine parameters were obtained in the fasted state. Serum was obtained by drawing blood into serum collection tubes (No. 03.1397, Sarstedt), incubating for 30 minutes on top of crushed ice to reduce variations caused by seasonal differences in room temperature, and centrifuging 10 minutes at 2000*g*, 4 °C. Aliquots were immediately prepared on ice and stored at −80 °C.

Proximity Extension Assay

Serum samples were analyzed with the OLINK Target 96 Inflammation panel (OLINK Proteomics) that cover 92 proinflammatory and anti-inflammatory cytokines, chemokines, growth factors, and factors involved in acute inflammatory and immune responses, angiogenesis, fibrosis, and endothelial activation. The assay allows for a relative quantification of analyte concentrations, which are given as normalized protein

expression (NPX) based on real-time quantitative polymerase chain reaction (qPCR) cycle values on a $\log_2$ scale (11). Only the 69 proteins with at least 80% of values above the limit of detection at one or more time points were used for analysis. OLINK assay data from one sample were missing. Two cytokines, IL6 and VEGFA, were also measured using ELISAs (R&D Systems; VEGFA catalog No. DVE00, Antibody Registry: AB_2800364; IL6 catalog No. HS600C, Antibody Registry: AB_2893335). NPX values (PEA) and log-transformed absolute protein concentrations (ELISA) were highly correlated both for IL-6 ($r = 0.86$, $P < .001$) and VEGFA ($r = 0.93$, $P < .001$) Supplementary Fig. S1 (16).

Whole-Blood RNA Isolation, Complementary DNA and Gene Expression Analysis

Whole blood was collected in PAXgene tubes after overnight fasting, and total cellular RNA was extracted using the PAXgene Blood RNA Kit (Qiagen). Subsequently, 1 µg of total RNA was reverse transcribed into complementary DNA (cDNA) using the Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics) according to the manufacturer's instructions and using an 1:1 mix of random hexamer primers in a volume of 20 µL. Real-time qPCR was performed using 1 µL of the resulting cDNA and the QuantiFast SYBR Green PCR Kit (Qiagen) according to the manufacturer's instructions in a total volume of 20 µL using the following QuantiTect Primer Assays (Qiagen): CXCL1 (QT02559186), CXCL5 (QT00095431), IL6 (QT00083720), OSM (QT00209286), TGFA (QT00033887), TNF (QT01079561), and TNFSF14 (QT01011682). Results were normalized using the housekeeper TBP (QT00000721). Concentrations was determined using the standard curve method. qPCR analysis was performed using the LightCycler 480 System and analyzed using LightCycler 480 Software (v.1.5.0). Two qPCR data outliers were detected by principal component analysis and removed.

Statistical Analyses

Analyses were performed using R4.0.2/RStudio (v1.3.959). OLINK data were processed in their original $\log_2$ format. Other data were tested for normality by Shapiro-Wilk test from the R package "stats" (v4.0.2), and nonnormal data were log-transformed. Paired *t* tests were used to compare cytokine responses between 2 time points and Pearson *r* was used for correlation analysis. Benjamini-Hochberg (BH) correction was used to account for multiple testing as indicated. Graphs were plotted using the R packages "ggplot2" (v3.3.5), "ggsignif" (v0.6.3), and "ggrepel" (v0.9.1).

Results

Study Population

The anthropometric, metabolic, and fitness parameters of the 22 (14 female and 8 male) participants from whom serum samples at the 2 acute exercise sessions were available are shown in Table 1. The participants were obese (BMI 31.7 ± 4.4) and untrained (less than 120 minutes structured exercise per week; VO_2peak 25.2 ± 4.2 mL/[kg × min]). Resting IL6 plasma levels quantified by ELISA were less than or equal to 4 pg/mL in all participants (mean 1.86 ± 0.85 pg/mL), indicating an absence of subclinical inflammatory processes. After the 8-week intervention, participants showed significant improvements in IAT, VO_2peak, and exercise performance, and significant reductions in body mass, BMI, total adipose tissue, and subcutaneous and visceral adipose tissue volume.

Acute Cytokine Response to Exercise

After the first acute exercise session, 40 out of the 69 included cytokines were increased more than 1.2-fold (BH-adjusted $P < .05$) (Fig. 1A, Table 2, and Supplementary Table S1) (16). The highest increases were found for oncostatin M (OSM) (2.31-fold), transforming growth factor α (TGFA) (2.07-fold), C-X-C motif chemokine ligand (CXCL) 5 (1.99-fold), TNF superfamily member (TNFSF)14 (1.93-fold), and CXCL1 (1.89-fold), all of which exhibited a BH-adjusted *P* less than .001. Several cytokines showed an increase of more than 1.5-fold. Interestingly, the prototypic exercise-regulated cytokine, IL6, was increased only 1.29-fold (BH-adjusted $P < .001$) (see Fig. 1A). The proinflammatory cytokine TNF was increased to a similar extent as IL6 (1.26-fold, BH-adjusted $P < .001$).

After the second acute exercise session, which was conducted after the 8-week training, 37 cytokines were increased more than 1.2-fold (BH-adjusted $P < .05$) (Fig. 1B, Table 2, and Supplementary Table S1) (16). The mean increase was highly comparable to the increase caused by the first, pretraining, exercise session (Fig. 1C). Again, OSM, TGFA, CXCL1 and 5, and TNFSF14 were the cytokines with the largest increase (> 1.9-fold, BH-adjusted $P < .001$). Comparing the response to the acute exercise session before and after the 8-week training revealed no statistically significant difference in the change in any of the 69 cytokines ($P > .05$ paired *t* test).

Acute Increase in Oncostatin M, Transforming Growth Factor α, and C-X-C Motif Chemokine Ligand 1 Transcripts in Cellular RNA From Whole Blood

An acute increase in circulating leukocytes, in particular NK cells, in response to exercise has been demonstrated that can contribute to an acute increase in cytokines in blood (17). We have no information about the acute regulation of the number of blood leukocytes in our study, but instead we analyzed the cellular transcripts of OSM, TGFA, and TNFSF14 together with CXCL1, CXCL5, TNF, and IL6 in whole-blood samples collected immediately after the acute exercise sessions together with the serum samples. The transcript levels of TNF and IL6 were unchanged, whereas OSM, TGFA, and CXCL1 transcripts were increased in whole blood both after the first and the last acute exercise session (Fig. 2). Notably, the leukocyte messenger RNA response was more pronounced after the 8-week training. The latter effect was also seen for CXCL5 and TNFSF14 transcripts, which were acutely upregulated in whole blood after the training period but not in untrained individuals. While the increase in transcript levels in whole blood cannot prove a direct contribution of circulating leukocytes to the increase in the respective cytokines in serum, a role for leukocytes is at least conceivable based on these data.

Effect of the 8-week Training Intervention on Cytokines in the Resting State

To assess potential training effects on circulating cytokine levels, the cytokine levels in the fasting blood samples taken before the exercise bouts were compared. Despite the clear effects of the intervention on fitness and adiposity, no change

Table 1. Anthropometric, fitness, and metabolic data

Parameter	Pre 8 wks	Post 8 wks	*P*
Sex	14 women/8 men		—
Age, y	30 ± 8.7 (19-59)	30.1 ± 8.65(19-59)	—
Height, cm	171 ± 9.47(157-193)		—
Body mass, kg	92.6 ± 16.1 (69.9-132)	91.4 ± 16.6 (67.2-130)	.008
BMI	31.7 ± 4.38 (27.5-45.5)	31.3 ± 4.58 (26.3-45.2)	.006
Waist to hip ratio	0.89 ± 0.06 (0.76-1.03)	0.89 ± 0.06 (0.74-0.98)	> .1
Total AT volume, L	40.4 ± 11.5 (25.3-74.2)	39.4 ± 11.6 (22.8-73.9)	.004
Subcutaneous AT, L	15.3 ± 5.73 (8.42-32.2)	14.7 ± 5.93(7.20-33.1)	.006
Visceral AT, L	3.53 ± 1.61 (0.81-7.26)	3.38 ± 1.54 (0.94-6.68)	.012
IAT_{ergo}/BM, W/kg	1.11 ± 0.22 (0.77-1.55)	1.32 ± 0.26 (0.89-1.87)	< .001
VO_2peak_{ergo}/BM, mL/(kg × min)	25.0 ± 4.15 (18.3-32.3)	26.5 ± 4.55(16.0-34.9)	.042
Performance cycling, W	119 ± 29.8 (67.5-180)	127 ± 24.9(77.5-175)	.011
Glucose fasting, mmol/L	5.09 ± 0.39 (4.61-6.00)	5.02 ± 0.39 (4.33-5.61)	> .1
Glucose $OGTT_{120\ min}$, mmol/L	5.70 ± 1.17 (3.44-8.00)	5.52 ± 1.58(3.50-11.6)	.089
Insulin fasting, pmol/L	110 ± 40.4 (38.0-188)	103 ± 36.9(50.0-170)	> .1
Insulin $OGTT_{120\ min}$, pmol/L	546 ± 400 (65.0-1766)	472 ± 341(61.0-1345)	.095
ISI_{Mats}	8.35 ± 4.90 (3.12-27.0)	8.86 ± 4.20(4.47-21.4)	> .1
HbA_{1c}, mmol/mol Hb	34.2 ± 2.41 (28.0-39.0)	33.5 ± 2.64(26.0-37.0)	.050
HbA_{1c},%	5.28 ± 0.22 (4.71-5.72)	5.22 ± 0.24 (4.53-5.54)	.050
Leukocytes, 1/µL	6890 ± 1513 (4740-10 200)	6485 ± 1437 (4220-9220)	> .1
Interleukin 6, pg/mL	1.86 ± 0.85 (0.77-4.06)	1.90 ± 1.12(0.77-5.84)	> .1

Paired *t* tests or Wilcoxon signed rank tests when data were not normally distributed. N = 22 or N = 21 for VO_2peak_{ergo}/BM, mean ± SD (range of values). Participants were a subgroup of the study published in Hoffmann et al (12).
Abbreviations: AT, adipose tissue; BM, body mass; BMI, body mass index; ergo, bicycle ergometer; HbA_{1c}, glycated hemoglobin A_{1c}; IAT, individual anaerobic threshold; ISI, insulin sensitivity index; ISI_{Mats}, insulin sensitivity index estimated by Matsuda method; OGTT, oral glucose tolerance test; VO_2peak, peak oxygen uptake.

in the 69 cytokines was observed in the resting serum values before and after the 8-week intervention (BH-adjusted $P > .05$) (see Table 2 and Supplementary Table S1) (16).

Effect of Adipose Tissue Compartments on the Acute Cytokine Response

Next, we performed exploratory correlation analyses to test whether individual cardiorespiratory fitness (quantified as VO_2peak) or metabolic parameters such as subcutaneous or visceral adipose tissue volume and systemic insulin sensitivity might have an influence on the acute cytokine response to exercise (Fig. 3). The fitness level did not correlate with any of the acute increases. BMI and visceral adipose tissue, but not subcutaneous adipose tissue volume, was positively correlated to the increase of several cytokines in the untrained participants before the intervention. After the 8-week training, the correlation remained for visceral adipose tissue and CXCL10 and sulfotransferase family 1A member 1 (ST1A1). Systemic insulin sensitivity assessed by the Matsuda insulin sensitivity index correlated negatively to the increase in IL6 and positively to ST1A1 before the intervention.

Increase in Interleukin-6 Correlates With Increase in Lactate

Last, we studied whether the increase of lactate was associated to the increase in cytokines. Blood lactate concentrations increase depending on the intensity of the performed ergometer exercise and correlate also with perceived exertion (18). In our study, plasma lactate concentrations were increased to a similar extend after both acute exercise sessions with no effect of the training intervention (3.87 and 3.82-fold; $P < .001$ paired *t* test) (Fig. 4A). Only IL6 was correlated to the increase in lactate after the first exercise bout ($P < .03$, $r = 0.48$) and the exercise bout after training ($P = .045$, $r = 0.43$) (Fig. 4B and 4C). The increase in CST5 ($P < .0085$, $r = 0.57$) and monocyte chemoattractant protein 1 (MCP1) ($P < .036$, $r = 0.47$) was correlated to the increase in lactate only after the first exercise bout.

Discussion

Proximity extension assay analysis of the acute cytokine response to exercise in overweight to obese individuals confirmed the increase in several cytokines and chemokines, chemokine receptor ligands, and MCPs that were previously reported in ELISA-based studies of lean and physically active humans (8). The physiological function of this increase in cytokines is to induce leukocyte chemotaxis, to activate T cells, to regulate adaptive immune responses, and to promote angiogenesis and smooth cell proliferation. Prototypical proinflammatory cytokines (TNF) showed only a minor increase compared with other cytokines or were below the limit of detection even after exercise (IL1α). Thus, our data underline the potential of exercise to modulate immune responses and to support anti-inflammatory pathways in a highly specific manner.

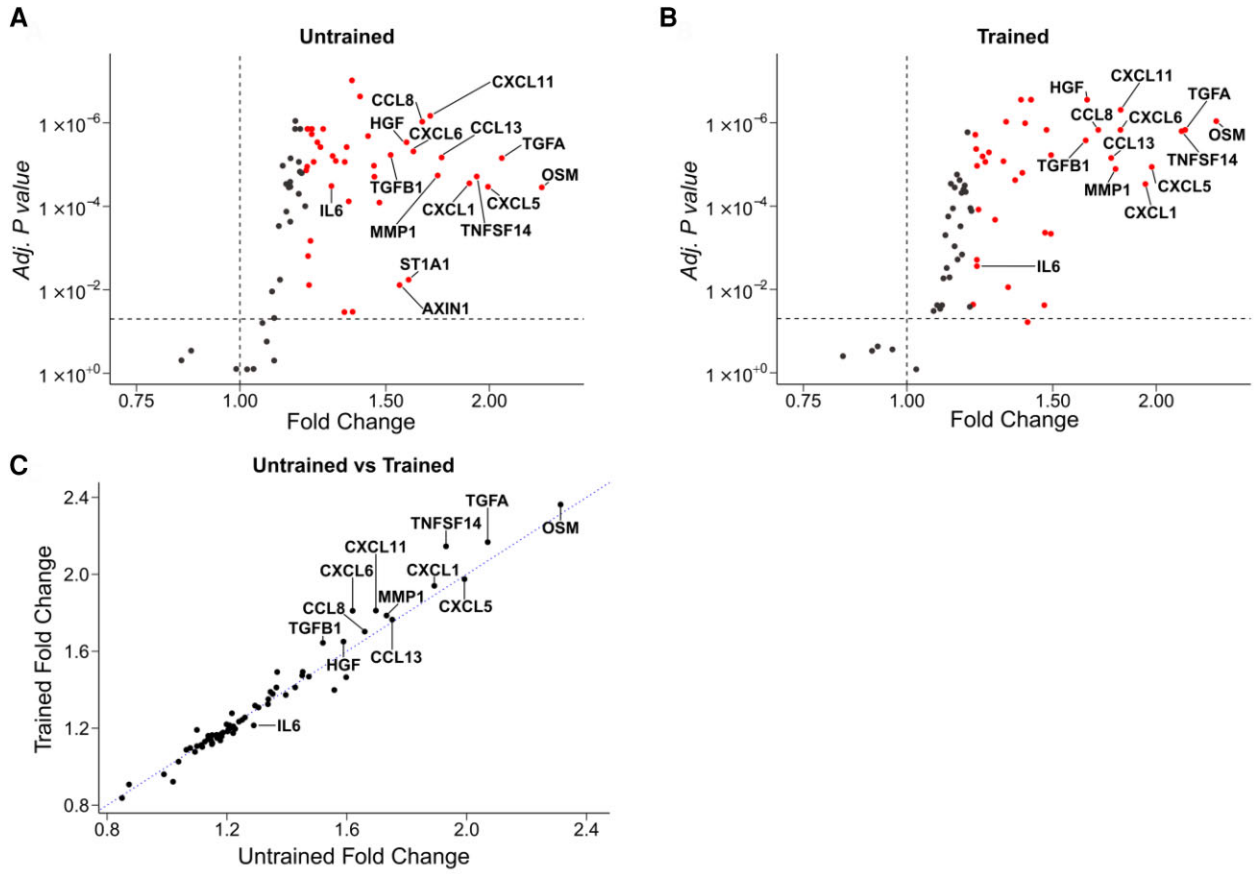

Figure 1. Acute exercise-induced changes in circulating cytokines. A and B, Volcano plots showing changes in serum levels of cytokines caused by 30 minutes of cycling in A, untrained individuals (n = 21) and B, after 8-week endurance training (n = 22). Red color highlights a fold change greater than 1.2. IL6 and all cytokines with a fold change greater than 1.5 are annotated. C, Correlation between acute exercise-induced cytokine changes before and after endurance training. The dotted line represents equality. *P* values were adjusted for multiple testing according to Benjamini-Hochberg.

In addition, our approach revealed a robust increase in cytokines that are yet not well characterized as exercise-regulated circulating proteins such as OSM, TGFA, and TNFSF14. OSM has been shown to increase in serum after one bout of 60-minute cycling in young and old individuals (19). OSM increased also in serum of mice after 60 minutes of swimming, which was paralleled by enhanced OSM transcript levels in skeletal muscle, but not in liver, adipose tissues, or spleen, leading to its denomination as a myokine (20). While OSM was also detected in the supernatant of human differentiated skeletal muscle cells by a cytokine antibody array (21), it is mainly expressed in immune cells including T cells, monocytes, macrophages, dendritic cells, granulocytes, and mast cells (22). The pronounced increase in OSM transcripts in cellular RNA isolated from whole blood immediately after exercise also argues for a contribution of circulating immune cells to the increase in serum OSM concentration. Of note, OSM and conditioned serum of exercised mice inhibit tumor cell growth and induce apoptosis (20), making it a promising candidate for exercise-related risk reduction in the development, progression, and recurrence of certain cancers (23). TNFSF14 is also widely expressed in immune cells (24), but only the regulation of TNFSF14 transcripts in whole blood in trained participants followed the pronounced increase in TNFSF14 protein after acute exercise. This might indicate an additional source of the circulating protein or different kinetics of transcript and protein regulation. TNFSF14, also known as LIGHT, has shown some potential in cancer immunotherapy (25), making it also a candidate for the therapeutic antitumor potential of exercise. TGFA showed a robust increase both in whole-blood transcript and in serum protein levels after acute exercise in untrained and trained participants, suggesting a contribution from leukocytes to the increase in circulating protein levels. TGFA has many mitogenic functions but less is known about its relevance as a systemic factor in the adaptation to exercise.

The overall response in cytokines to a 30-minute ergometer exercise bout before and after a structured and supervised 8-week endurance training was remarkably stable. Exercise intensity was individually set and monitored as the heart rate corresponding to 80% VO_2peak determined before the training period. As a consequence, absolute exercise performance assessed in watts was higher during the second 30-minute exercise bout compared to the first one whereas individual relative exercise intensity was unchanged. The constant relative exercise intensity was reflected by a similar increase in lactate before and after the training period. Thus, our results indicate that the increase in cytokines is proportional to the relative, rather than absolute, exercise intensity. The effect of training on the acute exercise–induced regulation of plasma proteins was also addressed in a recent study taking into account the improvement in exercise performance (26). When the second exercise bout after a 6-week endurance training period was performed at the same absolute intensity as the first

Table 2. Response of serum cytokines to acute exercise and training intervention

Protein	Untrained acute ex. adj. *P*	Untrained acute ex. FC+range	Trained acute ex. adj. *P*	Trained acute ex. FC+range	Training intervention adj. *P*	Training intervention FC+range	Protein	Untrained acute ex. adj. *P*	Untrained acute ex. FC+range	Trained acute ex. adj. *P*	Trained acute ex. FC+range	Training intervention adj. *P*	Training intervention FC+range
ADA	.047	1.10 (0.68-1.49)	.005	1.11 (0.85-1.38)	> .1	1.06 (0.62-1.51)	HGF	< .001	1.59 (1.00-2.53)	< .001	1.65 (0.94-2.4)	> .1	1.03 (0.52-2.04)
AXIN1	.047	1.56 (0.34-6.15)	.005	1.40 (0.21-3.50)	> .1	1.24 (0.16-9.23)	IFNG	< .001	0.99 (0.79-1.54)	< .001	0.96 (0.67-1.32)	> .1	1.00 (0.31-8.38)
CASP8	.008	1.21 (0.87-1.88)	.061	1.21 (0.81-1.79)	> .1	1.02 (0.55-1.80)	IL10	< .001	1.12 (0.73-1.49)	.003	1.10 (0.81-1.71)	> .1	1.06 (0.82-2.92)
CCL11	.002	1.35 (1.02-2.06)	.002	1.39 (0.96-1.94)	> .1	1.01 (0.66-1.77)	IL10RA	> .1	1.18 (0.96-1.51)	.029	1.14 (0.88-1.36)	> .1	0.97 (0.71-1.25)
CCL13	< .001	1.75 (1.17-5.44)	< .001	1.76 (0.86-3.73)	> .1	1.01 (0.31-3.83)	IL10RB	< .001	1.15 (0.93-1.34)	< .001	1.12 (0.90-1.38)	> .1	1.03 (0.85-1.31)
CCL19	< .001	1.21 (0.96-1.54)	< .001	1.19 (0.91-1.64)	> .1	1.03 (0.67-2.05)	IL12B	.006	1.15 (0.91-1.37)	.024	1.16 (0.89-1.52)	> .1	1.03 (0.75-1.49)
CCL2	> .1	1.35 (0.98-3.06)	.026	1.38 (0.71-2.06)	> .1	1.03 (0.56-2.67)	IL15RA	< .001	1.15 (0.95-1.46)	< .001	1.12 (0.85-1.40)	> .1	1.06 (0.84-1.37)
CCL20	< .001	1.10 (0.34-3.28)	< .001	1.19 (0.61-2.62)	> .1	0.91 (0.21-4.23)	IL17C	< .001	1.08 (0.75-1.74)	.003	1.10 (0.75-1.48)	> .1	1.05 (0.32-1.95)
CCL23	< .001	1.40 (1.07-1.92)	< .001	1.37 (0.99-1.93)	> .1	1.17 (0.73-1.82)	IL18	< .001	1.18 (0.88-1.44)	< .001	1.17 (0.86-1.49)	> .1	1.01 (0.83-1.35)
CCL25	< .001	1.20 (0.93-1.72)	< .001	1.22 (0.90-1.88)	> .1	0.97 (0.75-1.24)	IL18R1	.008	1.21 (0.95-1.44)	.023	1.21 (0.94-1.55)	> .1	0.98 (0.77-1.21)
CCL28	< .001	1.31 (0.93-2.06)	< .001	1.31 (0.95-1.85)	> .1	1.00 (0.64-1.47)	IL6	< .001	1.29 (0.86-1.90)	< .001	1.21 (0.78-2.10)	> .1	0.97 (0.51-2.38)
CCL3	< .001	1.22 (0.81-1.77)	< .001	1.28 (0.86-2.32)	> .1	1.01 (0.51-1.83)	IL7	< .001	1.47 (0.84-3.31)	< .001	1.47 (0.69-2.89)	> .1	1.02 (0.41-2.20)
CCL4	< .001	1.45 (0.98-2.42)	< .001	1.47 (0.92-2.32)	> .1	1.03 (0.43-2.3)	IL8	< .001	1.21 (0.87-2.24)	< .001	1.20 (0.59-2.17)	> .1	1.08 (0.64-2.20)
CCL8	< .001	1.66 (1.18-3.14)	< .001	1.70 (1.00-3.33)	> .1	0.99 (0.40-2.17)	LIFR	< .001	1.18 (0.99-1.42)	< .001	1.16 (0.89-1.46)	> .1	1.05 (0.81-1.43)
CD244	< .001	1.22 (0.95-1.44)	< .001	1.21 (0.91-1.52)	> .1	1.04 (0.85-1.33)	MMP1	<.001	1.73 (1.10-4.78)	< .001	1.79 (1.01-4.09)	> .1	0.94 (0.34-3.17)
CD274	< .001	1.19 (0.90-1.41)	< .001	1.18 (0.89-1.59)	> .1	1.00 (0.54-1.35)	MMP10	< .001	1.14 (0.94-1.43)	< .001	1.16 (0.90-1.59)	> .1	1.15 (0.77-2.86)
CD40	< .001	1.29 (1.00-1.82)	.002	1.32 (0.92-1.67)	> .1	1.05 (0.75-1.42)	OPG	< .001	1.21 (1.00-1.52)	< .001	1.22 (0.92-1.68)	> .1	1.00 (0.80-1.31)
CD5	< .001	1.22 (0.97-1.54)	< .001	1.17 (0.87-1.52)	> .1	1.04 (0.86-1.44)	OSM	< .001	2.31 (0.94-10.6)	< .001	2.36 (0.95-5.92)	> .1	1.15 (0.21-9.07)
CD6	< .001	1.25 (0.96-1.67)	< .001	1.24 (0.90-1.61)	> .1	1.00 (0.71-1.49)	PLAU	< .001	1.17 (1.00-1.37)	< .001	1.17 (0.89-1.86)	> .1	1.07 (0.88-1.33)
CD8A	.034	1.18 (0.9-1.48)	.009	1.15 (0.80-1.72)	> .1	1.04 (0.68-1.49)	S100A12	< .001	1.37 (0.15-2.94)	< .001	1.49 (0.60-2.69)	> .1	0.72 (0.05-2.63)
CDCP1	< .001	1.2 (0.87-1.61)	< .001	1.18 (0.98-1.47)	> .1	1.01 (0.81-1.34)	SCF	< .001	1.17 (0.99-1.33)	< .001	1.15 (0.97-1.40)	> .1	1.09 (0.86-1.67)
CSF1	< .001	1.13 (1.00-1.33)	< .001	1.13 (0.95-1.40)	> .1	1.00 (0.81-1.19)	SLAMF1	< .001	1.15 (0.93-1.45)	.005	1.13 (0.81-1.49)	> .1	1.03 (0.80-1.48)
CST5	< .001	1.34 (0.90-15.0)	< .001	1.32 (0.95-8.97)	> .1	0.98 (0.74-1.24)	ST1A1	.006	1.6 (0.40-6.61)	.024	1.47 (0.37-7.34)	> .1	1.21 (0.28-5.08)
CX3CL1	< .001	1.23 (0.98-1.62)	< .001	1.20 (0.89-1.61)	> .1	1.12 (0.93-1.51)	STAMBP	> .1	1.04 (0.22-3.10)	> .1	1.03 (0.36-3.67)	> .1	1.12 (0.28-3.77)
CXCL1	< .001	1.89 (1.00-5.05)	< .001	1.94 (0.98-6.04)	> .1	0.95 (0.22-4.07)	TGFA	< .001	2.07 (0.89-5.32)	< .001	2.17 (0.79-4.96)	> .1	1.04 (0.29-3.91)
CXCL10	< .001	1.34 (1.04-2.15)	< .001	1.35 (0.80-2.15)	> .1	1.16 (0.30-3.44)	TGFB1	< .001	1.52 (0.77-2.37)	< .001	1.64 (0.87-2.87)	> .1	0.99 (0.44-2.31)
CXCL11	< .001	1.7 (1.18-3.25)	<.001	1.81 (1.05-3.35)	> .1	1.07 (0.35-2.75)	TNF	< .001	1.26 (1.06-1.85)	< .001	1.26 (0.90-1.69)	> .1	1.06 (0.73-1.54)
CXCL5	< .001	1.99 (1.14-7.70)	< .001	1.98 (1.00-6.05)	> .1	1.05 (0.21-4.68)	TNFB	.011	1.15 (0.94-1.38)	.033	1.14 (0.91-1.54)	> .1	1.05 (0.82-1.44)
CXCL6	.034	1.62 (0.97-3.47)	< .001	1.81 (0.97-3.09)	> .1	0.99 (0.42-2.68)	TNFRSF9	< .001	1.09 (0.90-1.56)	< .001	1.08 (0.84-1.33)	> .1	1.03 (0.75-1.39)
CXCL9	> .1	1.24 (1.02-1.61)	> .1	1.23 (0.84-1.48)	> .1	1.21 (0.45-3.87)	TNFSF12	< .001	1.43 (1.04-2.23)	< .001	1.41 (0.94-1.88)	> .1	1.07 (0.64-1.85)
DNER	> .1	1.14 (0.97-1.34)	> .1	1.14 (0.97-1.44)	> .1	1.08 (0.89-1.26)	TNFSF14	.062	1.93 (0.77-5.56)	.024	2.15 (0.81-6.63)	> .1	0.98 (0.29-2.15)
EIF4EBP1	< .001	0.85 (0.09-5.53)	< .001	0.84 (0.13-10.4)	> .1	1.27 (0.23-8.92)	TRAIL	< .001	1.11 (0.87-1.34)	< .001	1.11 (0.91-1.44)	> .1	1.06 (0.77-1.26)
FGF19	< .001	0.87 (0.43-4.16)	< .001	0.91 (0.43-3.04)	> .1	0.8 (0.33-2.32)	TRANCE	< .001	1.06 (0.83-1.28)	.001	1.09 (0.83-1.49)	> .1	1.05 (0.73-1.51)
FGF21	> .1	1.02 (0.51-2.25)	> .1	0.92 (0.51-1.62)	> .1	1.04 (0.20-5.61)	VEGFA	< .001	1.45 (1.01-2.90)	< .001	1.49 (0.87-2.49)	> .1	0.98 (0.44-1.74)
FLT3LG	< .001	1.37 (1.05-1.75)	< .001	1.41 (1.08-2.00)	> .1	0.95 (0.67-1.20)							

FCs of protein abundance is given as mean (range of values). *P* values were adjusted for multiple testing based on Benjamini-Hochberg.
Abbreviations: acute ex, acute exercise; FC, fold change.

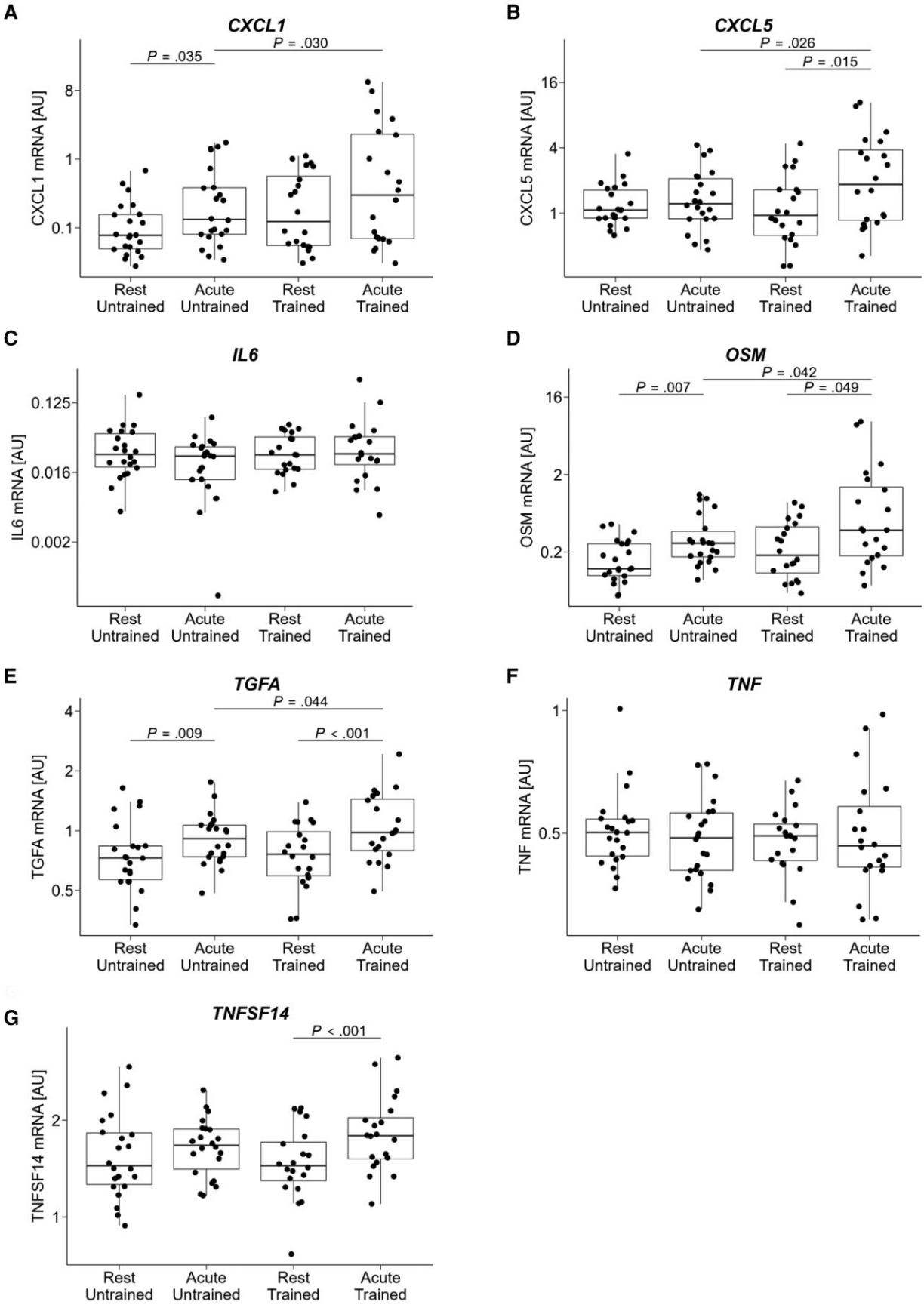

Figure 2. Transcript levels of cytokines in leukocytes. Box plots showing the expression of A, *CXCL1*; B, *CXCL5*; C, *IL6*; D, *OSM*; E, *TGFA*; F, *TNF*; and G, *TNFSF14* in leukocytes from untrained and trained individuals before and after acute exercise. Results were normalized using TBP transcript levels. The box represents the interquartile range (IQR, 25th to 75th percentile), and the black line represents the median. The whiskers extend the box to the lowest or largest value no further than 1.5 × IQR. AU, arbitrary units. N = 20-22.

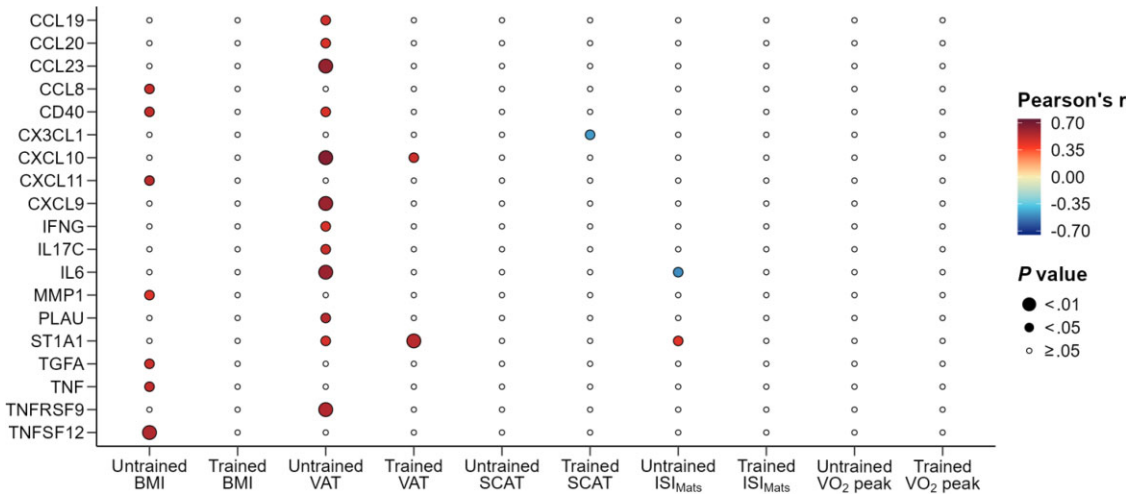

Figure 3. Correlation of acute exercise-induced changes in circulating cytokines with fitness and clinical data. The acute exercise-induced fold changes of serum cytokine concentrations in untrained or trained individuals were correlated to pretraining or posttraining body mass index (BMI), content of visceral adipose tissue (VAT), subcutaneous adipose tissue (SCAT), insulin sensitivity (ISI_{Mats}), and peak oxygen uptake (VO_2peak). The color illustrates the direction and strength of the correlation and the diameter indicates statistical significance; empty circles depict nonsignificant correlations. Only cytokines with at least one significant correlation are shown.

exercise bout before training, levels of some cytokines increased to a lesser extent, but this effect was not found when the intensity of the second acute exercise bout was adjusted to the improved fitness (26). Similarly, the increase in IL6 was reduced after 6 weeks of high-intensity interval running when comparing the response to one acute high-intensity interval running test at the same absolute workloads before and after training (27). When the workload of the acute exercise bout was adjusted to the improvement in maximal power output after 10 weeks of knee extensor endurance training, the acute response in plasma IL6 was similar before and after training (28). These results together with the data of our study clearly show that it is important for the comparison of the cytokine response to acute exercise to consider a potential training-induced change in the individual exercise performance.

This is even more evident when comparing the acute exercise response in individuals with different training status. The increase in IL6 and IL8 was not different between endurance-trained and sedentary young healthy males in a study of 30 minutes of treadmill running at 75% of the individual maximum oxygen uptake (VO_2max), albeit cardiorespiratory fitness was very different (VO_2max of 70 vs 47 mL/kg/min) (29). No data on the actual individual exercise performance were reported. Studying the response to a 60-minute ergometer test at 80% VO_2max in endurance-trained and sedentary young healthy males revealed that the trained individuals had even more pronounced increases in some of the analyzed proteins (eg, IL8, MCP1, IL10, TNF, TNFR2, MMP2, MMP3) and higher levels of the muscle damage markers creatine kinase and FABP after the exercise bout (30). Notably, exercise performance at 80%VO_2max was considerably higher in the trained group (254 vs 156 watts) despite a comparable heart rate in the trained and sedentary group (30). Thus, physical performance in relation to individual fitness is decisive for the cytokine response and an attenuated increase after training can be masked by the capacity to exercise at a higher absolute intensity.

In contrast to circulating protein, the transcript levels of CXCL1, CXCL5, OSM, TGFA, and TNFSF14 were higher after the acute exercise bout in the trained state compared to before the intervention. This might show a higher responsiveness in the expression of these genes in circulating leukocytes after training, which, however, was not translated to higher serum protein levels in samples collected at the same time point. We did not collect samples at a later time point, which would have allowed us to study whether the altered expression results in a prolonged increase of the respective cytokines in blood.

Another factor influencing the acute increase in several cytokines might be the visceral adipose tissue volume. We found a specific correlation for this fat compartment, but not for subcutaneous adipose tissue. Visceral adipose tissue has a higher content of NK cells, macrophages, and T cells, which is associated with a higher release of inflammatory cytokines (31). The mobilization or activation of the resident immune cells in visceral adipose tissue might contribute to the increase in serum cytokines in response to exercise. The correlation for most cytokines was not found after the 8-week training, except for CXCL10 and ST1A1. While this would argue for a training-induced adaptation of visceral adipose tissue immune cells resulting in a reduction in the release of certain cytokines after acute exercise, the comparable increase in cytokines before and after training and comparable resting values despite the reduction of visceral fat after training does not support the hypothesis. Further studies are needed to clarify the contribution of visceral fat to the cytokine response to exercise. The robust correlation of the increase in ST1A1 to visceral fat volume suggests the exercise-induced release of the protein from this compartment. The sulfotransferase ST1A1 is classified as intracellular protein, but was already detected by mass spectrometry–based proteomics in plasma (32, 33). Its function as secreted protein is unknown.

A clear result of our study is that the 8-week training intervention did not change the resting levels of cytokines related to inflammatory processes. While this would argue against the regularly described anti-inflammatory potential of training, it must be considered that our participants were on average obese but also relatively young and otherwise healthy.

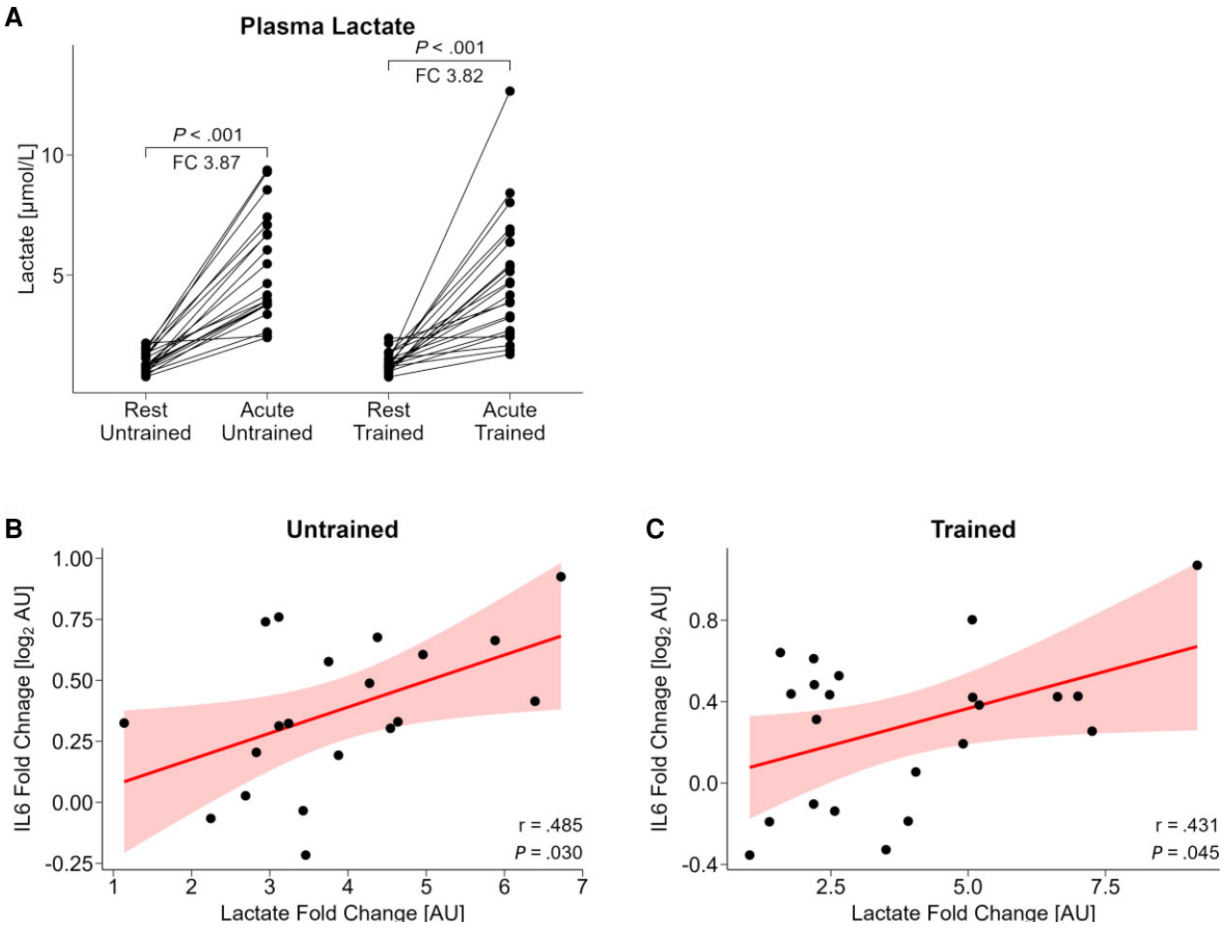

Figure 4. Lactate levels and correlation with interleukin 6 (IL6) after acute exercise. A, Plasma lactate concentrations before (rest) and after (acute) 30 minutes cycling in untrained individuals and after 8-week endurance training. B and C, Correlations between the fold changes in circulating IL6 and lactate in B, untrained and C, trained individuals. The shaded red area represents the 95% confidence level. AU, arbitrary units, FC, fold change. N = 21-22.

Resting IL6 plasma levels do not indicate subclinical inflammatory processes. Thus, despite considerably high BMI values, our participants could still have been too healthy to show a proinflammatory phenotype, which might explain why training could not alter their resting cytokine levels.

Previous studies have already described the association of the response of serum IL6 and lactate after acute exercise (34, 35), a correlation also reported for the release of IL6 and lactate from electrically stimulated human myotubes (34). Since the systemic increase in IL6 has also been linked to exercise duration and intensity and muscular glycogen depletion (36, 37), a common mechanism depending on muscular energy status might be assumed. In 2019, a study by Hojman et al (34) showed that the muscular production of lactate triggers the release of IL6 from intracellular vesicles involving the activity of matrix metalloproteases. This study validated previous results demonstrating the existence of preformed vesicles containing IL6 protein in skeletal muscle fibers allowing a rapid release of IL6 into the bloodstream from the contracting skeletal muscle (38). Our data support the concept of a specific, lactate-dependent regulation of the systemic levels of the myokine IL6, since only the increase in IL6, but not of any other analyzed cytokines, was correlated to the increase in lactate after both exercise bouts. We found this correlation despite an only moderate increase in serum IL6 after the 30-minute ergometer exercise bout.

There are limitations to our study. The increase in fitness and reduction in adiposity was moderate after the 8-week intervention and might not be sufficient to change resting cytokine levels or to modify the acute response. By design and as discussed, the participants performed the 2 acute exercise bouts at the same individual heart rate, which masked a potential reduction in the acute response of cytokines after training. Since the participants received a light standardized breakfast between the first blood draw and the commencement of exercise, we cannot exclude acute effects from food intake. However, data on alterations in systemic cytokine levels in the postprandial phase are not consistent (39) and food consumption or OGTT has been shown to acutely lower, rather than increase, IL-6 and have no effect on TNF levels in individuals with obesity (40, 41). Finally, due to the limited sample size, sex-specific differences were not be investigated in the present study.

In conclusion, our results provide a comprehensive insight into the acute cytokine response to one acute bout of exercise in overweight to obese individuals. Targeting a broad panel of cytokines strongly supports the immune modulatory function of exercise. We identified a number of still underexplored exerkines that showed a robust increase in serum and could

contribute to an adaptation mechanism to and clinical benefits from exercise.

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Author Contributions

T.G. and M.H. analyzed most of the data, interpreted the data, and wrote the manuscript with contributions from all coauthors; L.F. collected metabolic data; P.S. conduced exercise training; J.M. performed whole-body magnetic resonance imaging; A.P. and S.H. performed and analyzed proteomics data and provided scientific guidance; A.F., A.B., A.P., and M.H. provided scientific guidance and experimental design and contributed to discussions; A.N. designed the study and contributed to discussions; A.M. designed the study and analyzed metabolic data; and C.W. designed the study, supervised the whole project, interpreted the data, wrote the manuscript, and is guarantor of this work.

Disclosures

The authors have nothing to disclose.

Data Availability

The data are available from the corresponding author on reasonable request.

References

1. Cannon JG, Kluger MJ. Endogenous pyrogen activity in human plasma after exercise. *Science*. 1983;220(4597):617-619.
2. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund PB. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol*. 2000;529(Pt 1):237-242.
3. Lancaster GI, Febbraio MA. The immunomodulating role of exercise in metabolic disease. *Trends Immunol*. 2014;35(6):262-269.
4. Pedersen BK, Saltin B. Exercise as medicine—evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports*. 2015;25(Suppl 3):1-72.
5. Hoffmann C, Weigert C. Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations. *Cold Spring Harb Perspect Med*. 2017;7(11):a029793.
6. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol*. 2011;11(9):607-615.
7. Knudsen NH, Stanya KJ, Hyde AL, *et al*. Interleukin-13 drives metabolic conditioning of muscle to endurance exercise. *Science*. 2020;368(6490):eaat3987.
8. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev*. 2002;8:6-48.
9. Radom-Aizik S, Zaldivar F, Haddad F, Cooper DM. Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults. *J Appl Physiol (1985)*. 2013;114(5):628-636.
10. Zieker D, Fehrenbach E, Dietzsch J, *et al*. cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. *Physiol Genomics*. 2005;23(3):287-294.
11. Assarsson E, Lundberg M, Holmquist G, *et al*. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9(4):e95192.
12. Hoffmann C, Schneeweiss P, Randrianarisoa E, *et al*. Response of mitochondrial respiration in adipose tissue and muscle to 8 weeks of endurance exercise in obese subjects. *J Clin Endocrinol Metab*. 2020;105(11):e4023-e4037.
13. Dickhuth HH, Yin L, Niess A, *et al*. Ventilatory, lactate-derived and catecholamine thresholds during incremental treadmill running: relationship and reproducibility. *Int J Sports Med*. 1999;20(2):122-127.
14. Machann J, Thamer C, Schnoedt B, *et al*. Standardized assessment of whole body adipose tissue topography by MRI. *J Magn Reson Imaging*. 2005;21(4):455-462.
15. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9):1462-1470.
16. Goj T, Hoene M, Fritsche L, *et al*. Supplementary data for "The acute cytokine response to 30-minute exercise bouts before and after 8-week endurance training in individuals with obesity." Deposited November 11, 2022. https://figshare.com/articles/journal_contribution/supplemental_data_and_figure/21252333
17. Nieman DC, Henson DA, Austin MD, Brown VA. Immune response to a 30-minute walk. *Med Sci Sports Exerc*. 2005;37(1):57-62.
18. Scherr J, Wolfarth B, Christle JW, Pressler A, Wagenpfeil S, Halle M. Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. *Eur J Appl Physiol*. 2013;113(1):147-155.
19. Hwang JH, McGovern J, Minett GM, *et al*. Mobilizing serum factors and immune cells through exercise to counteract age-related changes in cancer risk. *Exerc Immunol Rev*. 2020;26:80-99.
20. Hojman P, Dethlefsen C, Brandt C, Hansen J, Pedersen L, Pedersen BK. Exercise-induced muscle-derived cytokines inhibit mammary cancer cell growth. *Am J Physiol Endocrinol Metab*. 2011;301(3):E504-E510.
21. Raschke S, Eckardt K, Holven KB, Jensen J, Eckel J. Identification and validation of novel contraction-regulated myokines released from primary human skeletal muscle cells. *PLoS One*. 2013;8(4):e62008.
22. Gorgens SW, Raschke S, Holven KB, Jensen J, Eckardt K, Eckel J. Regulation of follistatin-like protein 1 expression and secretion in primary human skeletal muscle cells. *Arch Physiol Biochem*. 2013;119(2):75-80.
23. Kim JS, Galvão DA, Newton RU, Gray E, Taaffe DR. Exercise-induced myokines and their effect on prostate cancer. *Nat Rev Urol*. 2021;18(9):519-542.
24. Kwon BS, Tan KB, Ni J, *et al*. A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. *J Biol Chem*. 1997;272(22):14272-14276.
25. Skeate JG, Otsmaa ME, Prins R, Fernandez DJ, Da Silva DM, Kast WM. TNFSF14: LIGHTing the way for effective cancer immunotherapy. *Front Immunol*. 2020;11:922.
26. Fonseca TR, Mendes TT, Ramos GP, *et al*. Aerobic training modulates the increase in plasma concentrations of cytokines in response

to a session of exercise. *J Environ Public Health*. 2021;2021: 1304139.
27. Croft L, Bartlett JD, MacLaren DP, *et al.* High-intensity interval training attenuates the exercise-induced increase in plasma IL-6 in response to acute exercise. *Appl Physiol Nutr Metab*. 2009;34(6): 1098-1107.
28. Fischer CP, Plomgaard P, Hansen AK, Pilegaard H, Saltin B, Pedersen BK. Endurance training reduces the contraction-induced interleukin-6 mRNA expression in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2004;287(6):E1189-E1194.
29. Landers-Ramos RQ, Jenkins NT, Spangenburg EE, Hagberg JM, Prior SJ. Circulating angiogenic and inflammatory cytokine responses to acute aerobic exercise in trained and sedentary young men. *Eur J Appl Physiol*. 2014;114(7):1377-1384.
30. Schild M, Eichner G, Beiter T, *et al.* Effects of acute endurance exercise on plasma protein profiles of endurance-trained and untrained individuals over time. *Mediators Inflamm*. 2016;2016: 4851935.
31. O'Rourke RW, Metcalf MD, White AE, *et al.* Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. *Int J Obes (Lond)*. 2009;33(9):978-990.
32. Geyer PE, Voytik E, Treit PV, *et al.* Plasma proteome profiling to detect and avoid sample-related biases in biomarker studies. *EMBO Mol Med*. 2019;11(11):e10427.
33. Keshishian H, Burgess MW, Gillette MA, *et al.* Quantitative workflow for sensitive biomarker discovery in plasma yields novel candidates for early myocardial injury. *Mol Cell Proteomics*. 2015;14(9): 2375-2393.
34. Hojman P, Brolin C, Nørgaard-Christensen N, *et al.* IL-6 release from muscles during exercise is stimulated by lactate-dependent protease activity. *Am J Physiol Endocrinol Metab*. 2019;316(5): E940-E947.
35. Minetto MA, Rainoldi A, Gazzoni M, Ganzit GP, Saba L, Paccotti P. Interleukin-6 response to isokinetic exercise in elite athletes: relationships to adrenocortical function and to mechanical and myoelectric fatigue. *Eur J Appl Physiol*. 2006;98(4):373-382.
36. Steensberg A, Febbraio MA, Osada T, *et al.* Interleukin-6 production in contracting human skeletal muscle is influenced by preexercise muscle glycogen content. *J Physiol*. 2001;537(Pt 2): 633-639.
37. Febbraio MA, Steensberg A, Keller C, *et al.* Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *J Physiol*. 2003;549(Pt 2):607-612.
38. Lauritzen HPMM, Brandauer J, Schjerling P, *et al.* Contraction and AICAR stimulate IL-6 vesicle depletion from skeletal muscle fibers in vivo. *Diabetes*. 2013;62(9):3081-3092.
39. Emerson SR, Kurti SP, Harms CA, *et al.* Magnitude and timing of the postprandial inflammatory response to a high-fat meal in healthy adults: a systematic review. *Adv Nutr*. 2017;8(2):213-225.
40. Manning PJ, Sutherland WH, Walker RJ, de Jong SA, Berry EA. The effect of glucose ingestion on inflammation and oxidative stress in obese individuals. *Metabolism*. 2008;57(10):1345-1349.
41. Manning PJ, Sutherland WH, McGrath MM, de Jong SA, Walker RJ, Williams MJ. Postprandial cytokine concentrations and meal composition in obese and lean women. *Obesity (Silver Spring)*. 2008;16(9):2046-2052.