

## Exercise-induced muscle-derived cytokines inhibit mammary cancer cell growth

Pernille Hojman,<sup>1,2\*</sup> Christine Dethlefsen,<sup>1\*</sup> Claus Brandt,<sup>1</sup> Jakob Hansen,<sup>1</sup> Line Pedersen,<sup>1</sup> and Bente Klarlund Pedersen<sup>1</sup>

<sup>1</sup>Centre of Inflammation and Metabolism at the Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen; and <sup>2</sup>Department of Oncology, Copenhagen University Hospital, Herlev, Denmark

Submitted 7 September 2010; accepted in final form 1 June 2011

**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* 301: E504–E510, 2011. First published June 7, 2011; doi:10.1152/ajpendo.00520.2010.—Regular physical activity protects against the development of breast and colon cancer, since it reduces the risk of developing these by 25–30%. During exercise, humoral factors are released from the working muscles for endocrinal signaling to other organs. We hypothesized that these myokines mediate some of the inhibitory effects of exercise on mammary cancer cell proliferation. Serum and muscles were collected from mice after an exercise bout. Incubation with exercise-conditioned serum inhibited MCF-7 cell proliferation by 52% and increased caspase activity by 54%. A similar increase in caspase activity was found after incubation of MCF-7 cells with conditioned media from electrically stimulated myotubes. PCR array analysis (CAPM-0838E; SABiosciences) revealed that seven genes were up-regulated in the muscles after exercise, and of these oncostatin M (OSM) proved to inhibit MCF-7 proliferation by 42%, increase caspase activity by 46%, and induce apoptosis. Blocking OSM signaling with anti-OSM antibodies reduced the induction of caspase activity by 51%. To verify that OSM was a myokine, we showed that it was significantly upregulated in serum and in three muscles, tibialis cranialis, gastrocnemius, and soleus, after an exercise bout. In contrast, OSM expression remained unchanged in subcutaneous and visceral adipose tissue, liver, and spleen (mononuclear cells). We conclude that postexercise serum inhibits mammary cancer cell proliferation and induces apoptosis of these cells. We suggest that one or more myokines secreted from working muscles may be mediating this effect and that OSM is a possible candidate. These findings emphasize that role of physical activity in cancer treatment, showing a direct link between exercise-induced humoral factors and decreased tumor cell growth.

breast cancer; physical activity; anti-cancer effect; myokines

ABOUT 25% OF CANCER CASES globally are due to excess weight and sedentary lifestyle (15). Contrarily, regular physical activity has been demonstrated to protect against various cancers. In 2007, the World Cancer Research Fund systematically reviewed the more than 50 studies regarding a possible association between physical activity and cancer and concluded that physical activity reduces the average risk for developing breast and colon cancers by 25–30% (28). These epidemiological findings led the World Cancer Research Fund to propose general recommendations of 30 min of daily moderate exercise. The anti-cancerous effects of physical activity are not yet

entirely understood, but the protective effects are believed to depend on the intensity, type, frequency, and duration of the activities (5, 27). The list of proposed mechanisms is long and includes changes in endogenous hormones, immune function, DNA repair, low-grade inflammation, and energy balance (8, 15, 17).

Research during the past 10 years has demonstrated that exercise induces the release of cytokines from working muscles (26). These muscle-derived cytokines are collectively named myokines, and the first myokine to be discovered was IL-6 (20, 24). However, recent research has demonstrated that skeletal muscles may produce and secrete numerous myokines belonging to distinctly different families (22). This highlights the fact that muscle tissue has an inherent ability to work as a secretory organ. The myokines have been studied extensively for their role in metabolism, e.g., as mediators of muscle-to-fat cross-talk and in controlling muscular glucose and fat metabolism (9, 14, 19, 22).

However, myokines may also play a role in cancer protection. Recently, it was shown that conditioned media from electrostimulated C<sub>2</sub>C<sub>12</sub> muscle cells could inhibit mammary cancer cell proliferation (3), suggesting that the contracting myotubes release humoral factors that can inhibit cancer cell growth. In continuation, Barnard et al. (2) have shown that serum obtained from subjects after 2 wk of a low-fat, high-fiber diet and exercise intervention could inhibit mammary cancer cell growth in vitro. Similar interventions in men were shown to inhibit prostate cancer cell proliferation (25).

The establishment of cancer is dependent on several factors, including immune reactivity toward neoplastic cells and growth factors. In this study, we hypothesize that factors secreted from muscles during physical activity have a direct effect on the cancer cells by inhibiting their proliferation, and thus physical activity, in addition to the effects on reduced adiposity, estrogen, and glucose levels, mediates independent protective effect against cancer through the release of antiproliferative myokines from working muscles.

### MATERIALS AND METHODS

**Cells and reagents.** The human mammary cancer cell line MCF-7 (estrogen receptor positive) was grown at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium containing (DMEM) 4.5 mg/l D-glucose and supplemented with 10% fetal bovine serum (FBS; Invitrogen) or 10% postexercise serum and 1% penicillin-streptavidine (Invitrogen). For incubation studies, the cytokines mouse recombinant GDF5 and human recombinant oncostatin M (OSM) (both R & D Systems) and human recombinant IL-10 (ImmunoTools) were used at 10 ng/ml. Mouse recombinant GDF5 was used since no human recombinant protein was available.

\* These authors have contributed equally to this article.

Address for reprint requests and other correspondence: P. Hojman, Centre of Inflammation and Metabolism, Dept. of Infectious Diseases, Rigshospitalet, Univ. of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark (e-mail: Pernille.Hojman.Moeller@rh.regionh.dk).

**Mouse exercise trial and PCR arrays.** All animal experiments were conducted in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimentation and after permission from the Danish Animal Experiments Inspectorate. Ten-week-old Naval Medical Research Institute mice (own breed) swam in 37°C warm water for 60 min and were euthanized immediately after, 2 h, or 5 h after the swimming bout. Control mice did not swim but were otherwise treated similarly to the exercising mice. For instance, food was withdrawn 1 h before euthanasia. Blood was collected by decapitation, and the tibialis cranialis, gastrocnemius, and soleus muscles, liver, spleen, and subcutaneous and visceral adipose tissues were quickly excised and frozen on dry ice and 100% alcohol. Sample size is 8 for serum and all tissue samples.

Total RNA was isolated from frozen tissues by tissue homogenization and RNA extraction using the TRIzol reagent (Invitrogen Life Technologies), and cDNA synthesis was performed using the RT2 PCR Array First Strand kit (SABiosciences). Custom-made PCR arrays (CAPM-0838E; SABiosciences) containing predeveloped reactions for 28 customized genes and four housekeeping genes were performed according to the manufacturer's instructions. N was 10 for each condition (pre-exercise, 0H, and 2H). Data analysis was performed using the web-based analysis tool ([www.sabiosciences.com/pcrarraydataanalysis.php](http://www.sabiosciences.com/pcrarraydataanalysis.php)), which includes descriptive statistics, including fold change, volcano plots, and cluster analyses. For OSM expression, RT-PCR was performed using random p(dN)<sub>6</sub> primers (Applied Biosystems) and MultiScribe Reverse Transcriptase (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems). All amplifications were done using the Taqman SYBR green PCR Master Mix (Applied Biosystems) and sequence-specific primers.

**Electrical stimulation of C<sub>2</sub>C<sub>12</sub> muscle cells.** Murine C<sub>2</sub>C<sub>12</sub> muscle cells were grown in triplets in six-well plates at a starting concentration of  $2 \times 10^4$  cells/well. When plates were confluent, differentiation was initiated by switching to differentiation medium 1 (DMEM, glutamax, low glucose, 10% FBS) followed by differentiation medium 2 (DMEM, high glucose, 2% HS). When cells were fully differentiated, electrical stimulation (2-ms duration, 40.0 V, 1 Hz) was performed on half of the wells, whereas the other half was nonstimulated controls. Media were changed before the stimulation and collected after 3 h of stimulation/nonstimulation and frozen at -20°C.

**Cell culture assays.** Serum from the exercising mice was pooled within the groups, followed by heat inactivation at 56°C for 30 min and sterile filtration. Cells were grown in triplets in six-well plates at a starting concentration of 100,000 cells/well and with 2.5 ml of medium containing 10% conditioned serum instead of FBS or 10 ng/ml recombinant protein.

A Caspase/CPP32 Colorimetric Assay kit (Biovision) was used to assay the activity of caspase. After incubation (24 h for postexercise serum experiment and 96 h for recombinant proteins) the cells were counted, and 1,000,000 cells from each well were spun down. The supernatant was removed, and cells were resuspended in 50  $\mu$ l of chilled cell lysis buffer (Biovision) and incubated on ice for 10 min. Following centrifugation, the cytosolic extract was transferred to a fresh tube and put on ice. Fifty micrograms of protein was diluted to 50  $\mu$ l of cell lysis buffer, followed by the addition of 50  $\mu$ l of 2 $\times$  reaction buffer (containing 10 mM DTT) and 5  $\mu$ l of 4 mM DEVD-pNA substrate (200  $\mu$ M final concentration). The plate was incubated at 37°C for 1 h, and absorbance was read at a microtiter plate reader at 405 nm.

An Annexin V-Biotin Apoptosis Detection kit (Biovision) was used to determine translocated phosphatidylserine. After incubation with the cytokines,  $5 \times 10^5$  cells were collected from each group and resuspended in 200  $\mu$ l of binding buffer (kit), and 5  $\mu$ l of Annexin V-biotin and 5  $\mu$ l of propidium iodid were added, followed by 5 min of incubation in the dark. Following washing, the cells were fixed in 2% formaldehyde (Bie & Berntsen) in phosphate-buffered saline (PBS; Invitrogen) and washed again in PBS. The cells were resuspended in 100  $\mu$ l of PBS with 1 mg/ml BSA and 5  $\mu$ l streptavidine

FITC (ElectraBox). After the final wash, the cells were resuspended in 200  $\mu$ l of PBS and analyzed by flow cytometry.

**Caspase activity in MCF-7 cells incubated with conditioned media.** MCF-7 cells were plated in six-well plates at concentration 60,000 cells/well. The next day, the cells were added to medium (+10% FBS) from the electrical stimulated/non-stimulated C<sub>2</sub>C<sub>12</sub> cells, and half of the wells were added to anti-OSM antibodies (R & D Systems). Additionally, control cells were grown in normal growth medium. All stimulations were performed three times in triplets. After 24 h, caspase activity was determined by the use of a caspase assay, as described earlier.

**Serum OSM and metabolite measurements.** Serum was collected from control mice ( $n = 8$ ) or immediately after 1 h of swimming ( $n = 10$ ) and stored at -80°C until assayed. Blood glucose was determined by Hemocue glucose 201 measurements. Enzyme-linked immunosorbent assay kit (Uscnk Life Science) was used for the quantitative measurement of mouse OSM according to the manufacturer's protocol. Meso Scale Discovery 3-plex kit was for used for quantitative analyse of serum insulin, glucagon, and glucagon-like peptide-1 (GLP-1).

**Statistics.** One-way ANOVA was used to compare the groups, and ad hoc posttesting by *t*-test with Bonferroni correction for multiple testing was used to determine differences among the individual groups. Two-way ANOVA was used to compare groups and time points in the swimming experiment. This analysis was also followed by ad hoc posttesting with Bonferroni correction to determine differences among the individual groups. A *P* value of <0.05 was considered to be statistically significant.

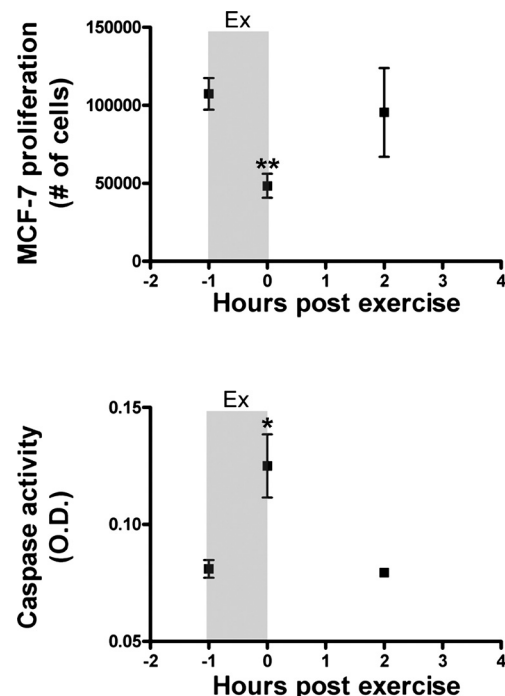


Fig. 1. Proliferation and caspase activity in MCF-7 cells stimulated with exercised serum (Ex). Serum was pooled for 10 mice taken pre-exercise (control), immediately after 1 h of swimming (0H), or 2 h into recovery after 1 h of swimming (2H). The starting concentration was 100,000 cells/well for all conditions. *Top*: no. of MCF-7 cells after 24 h of incubation with exercised serum. *Bottom*: caspase activity [optical density (OD)] after 24 h of incubation with exercised serum. \* and \*\*Statistical significance at  $P < 0.05$  and  $P < 0.001$ , respectively ( $n = 3$ ).

## RESULTS

*Exercise-conditioned serum inhibits mammary cancer cell proliferation and induces apoptosis.* Mice were euthanized before, immediately after, or 2 h into recovery after a 1-h swimming exercise bout, and serum was collected. Incubating MCF-7 cells with media containing 10% of this exercise-conditioned serum resulted in a 52% reduction in cell proliferation, with the serum collected immediately after swimming ( $P < 0.005$ ; Fig. 1, *top*). Serum taken 2 h into recovery had no effect on the proliferation rate. Similarly, caspase activity was analyzed in the cells incubated with the exercise-conditioned serum. Serum taken immediately after swimming increased caspase activity by 54% compared with resting levels ( $P < 0.05$ ), whereas serum taken 2 h into recovery had little effect on caspase activity (Fig. 1, *bottom*).

*Identification of exercise-induced myokines.* To identify which potential myokines were expressed in the working muscles, we performed PCR array analysis on 28 selected genes. These were chosen on the basis of previous reports that had proposed them as potential myokines. We found that seven of the 28 selected genes were significantly upregulated, and none were downregulated immediately after swimming (Fig. 2). The upregulated genes included OSM, IL-6, IL-11, GDF2, GDF5, CSF2, and IL-10. Two hours into recovery, IL-6, IL-11, GDF2, and GDF5 remained significantly upregulated (Fig. 2).

*Control of tumor cell growth by individual myokines.* We chose to investigate OSM, IL-10, IL-11, and GDF5 in detail. This selection was based on the availability of the candidates as recombinant proteins, and furthermore, we chose not to investigate IL-6 since numerous reports suggest that IL-6 does not possess an anti-cancer effect (16, 18). OSM and IL-10 in-

creased immediately after swimming but returned to baseline level after 2 h, whereas IL-11 and GDF5 remained significantly elevated both immediately and 2 h into recovery.

Adding these factors to the cell media at a concentration of 10 ng/ml, we determined cell proliferation and induction of apoptosis as measured by caspase activity and annexin-streptavidin staining. Incubation of MCF-7 cells for 5 days showed growth inhibitory effects of OSM ( $P < 0.05$ ) and a borderline decrease in growth by IL-10 ( $P = 0.07$ ) but no effect of IL-11 and GDF5 on MCF-7 proliferation, and these two candidates were not investigated further (Fig. 3). Pooling of the cytokines also showed a significant reduction in cell number after 5 days of incubation ( $P < 0.001$ ; data not shown). The apoptosis assays showed a significant upregulation ( $P = 0.034$ ) in caspase activity in MCF-7 cells stimulated for 96 h by OSM compared with the control group (Fig. 4, *top*). Similarly, annexin-streptavidin staining showed a significant increase in the number of apoptotic cells after 96 h of incubation with OSM compared with the control group (Fig. 4, *middle*). No effect of IL-10 on caspase activity or annexin-streptavidin staining was observed (data not shown).

To further assess whether OSM was responsible for the induction of caspase activity in stimulated MCF-7 cells, we electrically stimulated murine C<sub>2</sub>C<sub>12</sub> muscle cells to obtain a pure pool of muscle-derived myokines in response to an exercise stimuli. We then incubated the MCF-7 cells with the conditioned media from the muscle cells with or without anti-OSM antibodies. In line with the results from postexercise serum incubation studies, media from electrically stimulated muscle cells induced a 100% increase in caspase activity ( $P = 0.01$ ), whereas the presence of anti-OSM antibodies reduced this increase to a 49% increase compared with incubation with

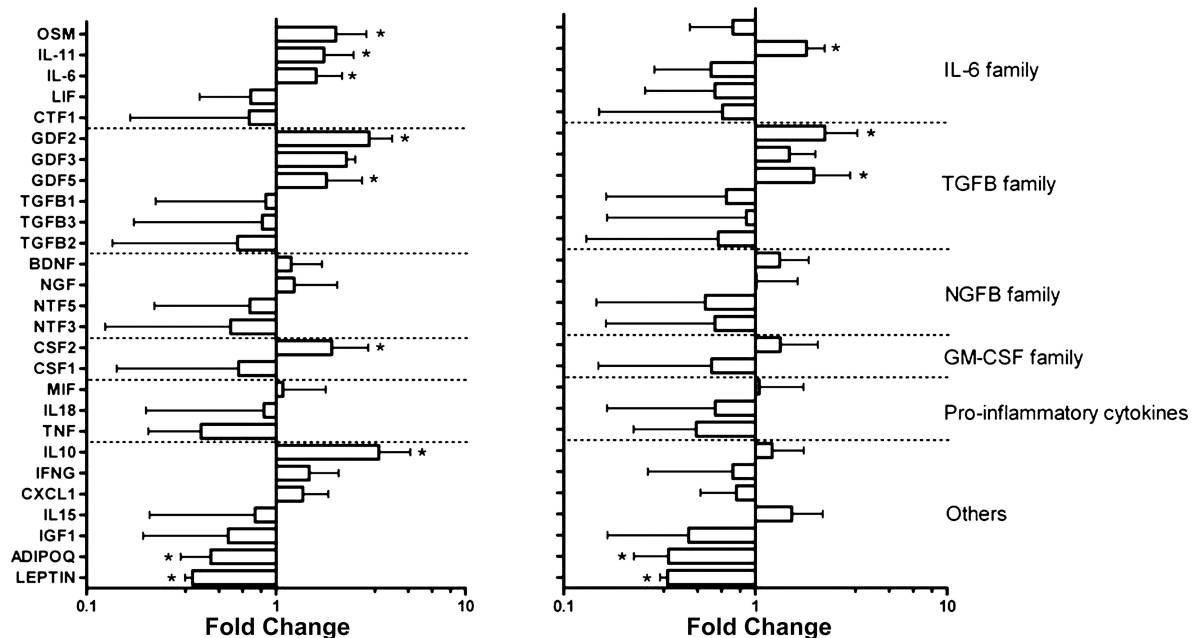


Fig. 2. Fold change in gene expression of selected myokines after swimming. Muscular expression of myokines in tibialis cranialis muscles is depicted as fold change compared with control mice. *Left*: immediately after swimming. *Right*: 2 h into recovery after swimming. Bars indicate means  $\pm$  SE ( $n = 10$ ). \*Significant fold changes ( $P < 0.05$ ). OSM, oncostatin M; CTF1, cardiotrophin 1; BDNF, brain-derived neurotrophic factor; NTF3 and -5, neurotrophin 3 and 5, respectively; GM-CSF, granulocyte-macrophage colony-stimulating factor; MIF, migration inhibitory factor; IFNG, interferon- $\gamma$ ; CXCL1, chemokine C-X-C motif; adipoQ, adiponectin.



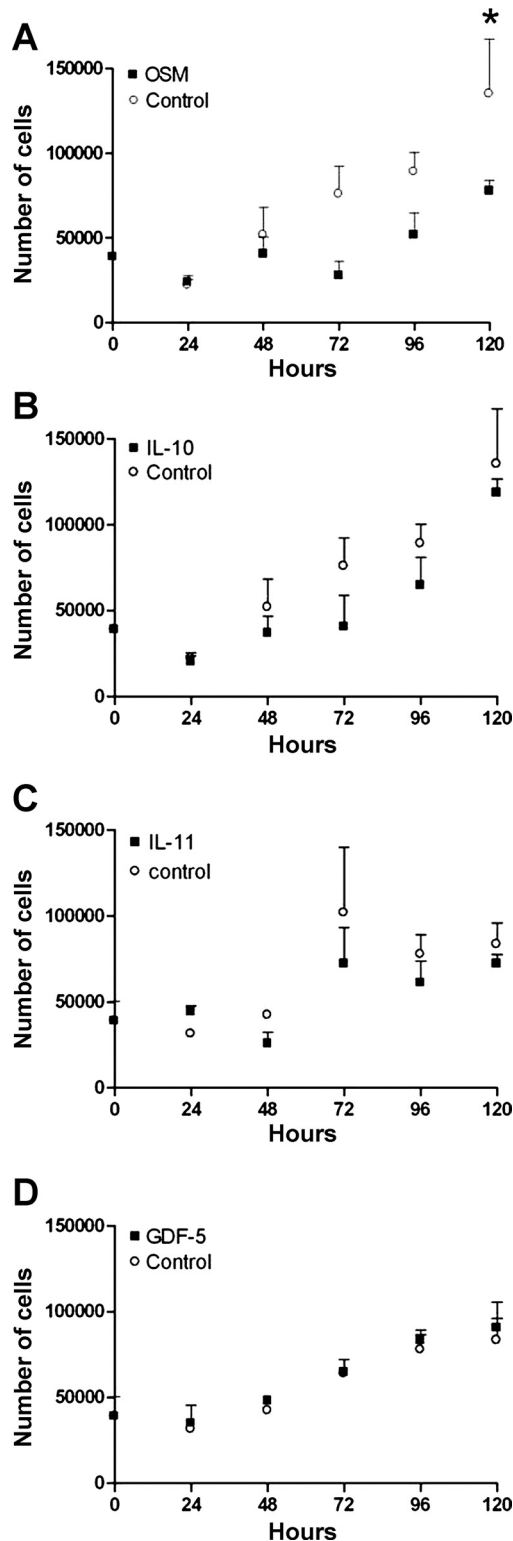


Fig. 3. Incubation of MCF-7 cells with different cytokines for 5 days. A: OSM (■) and control (○). B: IL-10 (■) and control (○). C: IL-11 (■) and control (○). D: GDF-5. All cytokines were added at a concentration of 10 ng/ml. \*Significance at  $P < 0.05$  ( $n = 3$ ).

conditioned media from nonstimulated myotubes ( $P = 0.1$ ) (Fig. 4, bottom).

*Is OSM a myokine?* Because OSM proved to be the most efficient cytokine, we measured the serum levels of OSM

following the exercise intervention. At the control level, serum OSM was expressed very weakly and was detectable in only four of eight mice (Fig. 5). After 1 h of swimming, OSM increased significantly ( $P < 0.001$ ) and was detectable in nine of nine mice. Two hours into recovery, serum OSM had returned to control levels. To verify the source of systemic OSM, we determined OSM expression in different tissues (Fig. 5). Following the swimming exercise bout, OSM expression was upregulated in all three muscles investigated, i.e., tibialis cranialis (4-fold,  $P < 0.001$ ), gastrocnemius (2-fold,  $P < 0.05$ ), and soleus (2-fold,  $P < 0.05$ ). OSM expression did not change in spleen (mononuclear cells), liver, or subcutaneous and visceral adipose tissue following the exercise bout, strongly suggesting that muscle tissue is the primary source of OSM.

## DISCUSSION

Our main finding is that exercise-conditioned serum is capable of inhibiting mammary cancer cell proliferation and inducing apoptosis through caspase activation. The direct use

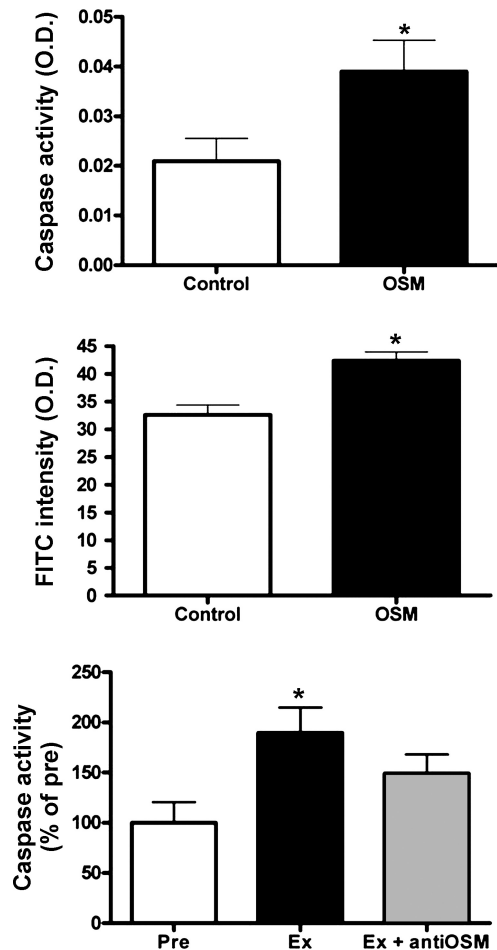


Fig. 4. Apoptosis assays in MCF-7 cells stimulated with OSM. MCF-7 cells were incubated for 96 h with OSM at 10 ng/ml as well as an untreated control. Top: caspase activity. Middle: annexin-streptavidin staining (mean FITC intensity). Bottom: caspase activity in MCF-7 cells incubated with media from electrically stimulated C<sub>2</sub>C<sub>12</sub> muscle cells with or without anti-OSM antibodies; Pre (media from nonstimulated C<sub>2</sub>C<sub>12</sub> cells), Ex (media from electrically stimulated C<sub>2</sub>C<sub>12</sub> cells), and Ex + anti-OSM (media from electrically stimulated C<sub>2</sub>C<sub>12</sub> cells with anti-OSM antibodies). \*Statistical significance at  $P < 0.05$  compared with control levels ( $n = 8$ ).

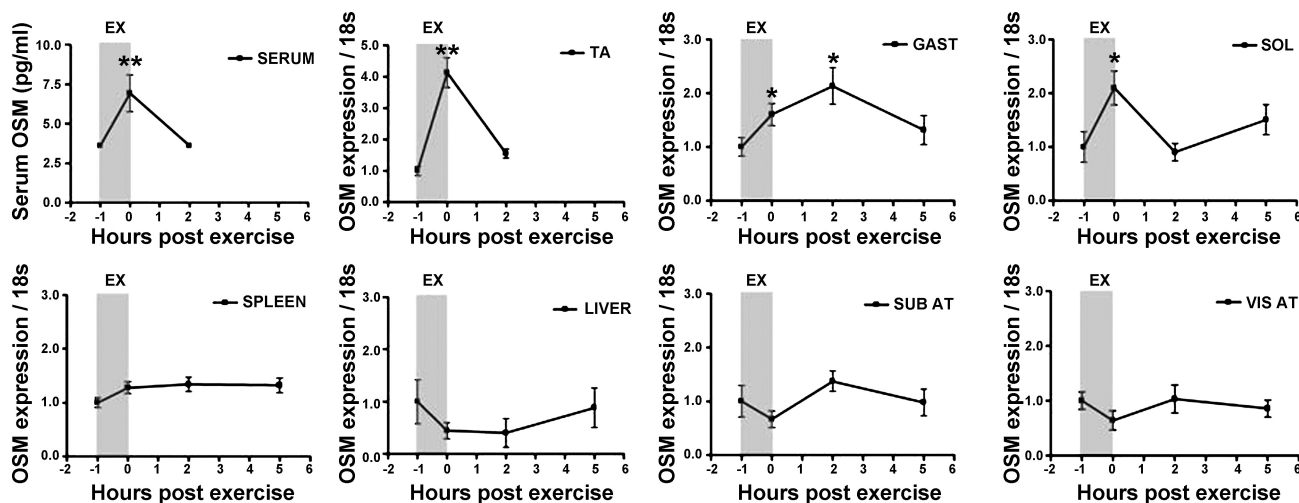


Fig. 5. OSM serum concentration and tissue expression after 1 h of swimming. OSM was measured in serum samples taken from control mice ( $n = 8$ ) or immediately after 1 h of swimming ( $n = 10$ ). OSM mRNA expression was determined in the tibialis cranialis muscle (TA), gastrocnemius muscle (GAST), soleus muscle, spleen, liver, subcutaneous adipose tissue (SUB AT), and visceral adipose tissue (VIS AT) before, immediately after 1 h of swimming, and 2 and 5 h into recovery after the exercise bout. Data are depicted as OSM expression normalized to pre-exercise value ( $n = 8$  for all tissues). \* and \*\*Statistical significance at  $P < 0.05$  and  $P < 0.001$ , respectively.

of exercise-conditioned serum in this study bypasses *in vivo* modulations like the immune system and hypoxia and emphasizes the role of systemic factors, which change during an exercise bout. Our results clearly suggest that muscle-derived humoral factors (myokines) can inhibit tumor cell growth and that at least some of the antiproliferative response of physical activity is due to a direct release of humoral factors to the circulation during exercise bouts.

Our approach was to incubate mammary cancer cells with conditioned serum from exercising mice. A similar approach was undertaken by Barnard et al. (2), who conducted studies with obese postmenopausal subjects and collected serum 24 h after the exercise intervention. In these studies, the exercise intervention was combined with a low-fat, high-fiber diet for 2 wk, and thus the effect of the metabolic profile in the serum caused by the diet or exercise intervention could not be differentiated. The intervention was associated with reductions in serum estradiol, insulin, and IGF-I and proved to significantly reduce MCF-7 proliferation (2). In our study, we found that acute changes in serum could inhibit mammary cancer cell proliferation. At this time point, the exercise-induced myokines are at the highest level. In addition, we observed reductions in serum insulin (Table 1), whereas serum estradiol was not investigated since these studies were performed in male mice.

To identify potential muscle-derived factors that could explain the reduction in cancer cell proliferation, we performed a partial expression profile screening using PCR arrays. In this, we included genes encoding 28 candidate myokines that rep-

resent known and potential myokine families, i.e., IL-6 superfamily, transforming growth factor- $\beta$  family, NGF $\beta$  family, and other miscellaneous candidates, which have been suggested to play a role in muscle-to-fat cross-talk (21). In continuation of this selection, seven genes were upregulated acutely after 1 h of swimming, whereas none were downregulated. Several of the genes remained elevated 2 h after the termination of swimming, including IL-11, GDF-2, and GDF5, whereas others returned to baseline expression, i.e., OSM and IL-10.

OSM proved to be of particular interest because this cytokine significantly inhibited MCF-7 cell proliferation and induced apoptosis. Moreover, blockade of OSM signaling by addition of anti-OSM antibodies halved the induction of caspase activity. We also found that OSM increased significantly from nearly undetectable levels to significant serum concentrations immediately after the exercise bout and declined 2 h after the exercise bout. Of note, only serum obtained immediately after the exercise, but not 2 h after, had inhibitory effects on cancer cell growth. This serum increase was paralleled by an increase in the muscular expression of OSM in all three muscles investigated. This implies that muscle tissue is the primary source of OSM during exercise and that OSM can be considered as a myokine. Interestingly, the concentration of OSM goes from undetectable levels at resting conditions to measurable picogram per milliliter levels immediately after exercise, suggesting that OSM works even at very low concentrations. Previous studies have shown a dose-dependent inhibition on MCF-7 cells with OSM (4, 12). Doses down to 400 pg/ml

Table 1. Blood glucose, serum insulin, glucagon, and GLP-1 values measured in the mice from the swimming experiment

|                       | Pre-Exercise        | 0 h                  | 2 h                | 5 h                  |
|-----------------------|---------------------|----------------------|--------------------|----------------------|
| Blood glucose, mmol/l | 8.1 $\pm$ 0.2       | 8.0 $\pm$ 0.7        | 6.35 $\pm$ 0.3*    | 6.35 $\pm$ 0.3*      |
| Insulin, pg/ml        | 2,242.7 $\pm$ 239.9 | 1,299.2 $\pm$ 256.6* | 540.1 $\pm$ 112.2* | 1,084.1 $\pm$ 307.9* |
| Glucagon, pg/ml       | 337.5 $\pm$ 36.8    | 363.8 $\pm$ 15.0     | 362.3 $\pm$ 14.2   | 311.1 $\pm$ 12.3     |
| GLP-1, pg/ml          | 27.1 $\pm$ 1.6      | 29.2 $\pm$ 1.1       | 22.3 $\pm$ 2.0     | 22.1 $\pm$ 1.8       |

Values are means  $\pm$  SE. GLP-1, glucagon-like peptide-1. \*Statistical significance at  $P < 0.05$  compared with pre-exercise levels ( $n = 8$ ).

have been shown to have inhibitory effects in in vitro systems, whereas the main effect occurs above 2 ng/ml. Our serum OSM increases following exercise might not reach these levels, but we believe that OSM still plays a role in our system since addition of anti-OSM antibodies to conditioned media from electrically stimulated C<sub>2</sub>C<sub>12</sub> cells can partly blunt the induction of caspase activity in the MCF-7 cells.

OSM is a pleiotropic cytokine belonging to the IL-6 superfamily, and studies suggest that OSM is expressed in 66% of breast tumors, with the prevalence being even higher in inflammatory breast cancers (6). The antiproliferative role of OSM has been documented in many in vitro studies, and in line with our results, OSM has been shown to inhibit proliferation of tumor cell lines derived from a variety of tissues, including ovarian, lung, human cerebral meningioma, mammary epithelial cells, and melanoma cells (7, 10, 13). However, following the initial interest in OSM for its antiproliferative effects, it has become increasingly apparent that the effects of OSM are also associated with breast cancer cell detachment and angiogenesis (11).

We also tested IL-10, IL-11, and GDF5 for their antiproliferative effects. Regarding IL-10, a large and contradicting literature exists proving that IL-10 can both inhibit and stimulate tumor cell growth. We found that IL-10 tended to reduce the proliferation of MCF-7 cells, whereas it had no effect on induction of apoptosis. Little evidence on the role of IL-11 and GDF5 on tumor proliferation exists, and we found no effect of IL-11 GDF5 on the proliferation of MCF-7.

In addition to the myokines that were induced by exercise, the swimming exercise bout also tended to decrease a number of proinflammatory cytokines and growth factors that have been shown to play a role in tumor progression, including TNF $\alpha$ , IGF-I, and leptin (1, 23). Although not significant, the reduction in muscular expression was seen to last at least 2 h into recovery. We measured TNF $\alpha$ , IL-6, and monocyte chemoattractant protein-1 in the serum samples, but none of these proinflammatory cytokines were detectable either before or after the swimming bout (data not shown). Furthermore, we measured insulin, glucagon, and GLP-1 (Table 1). We found that insulin decreased after the exercise bout and remained depressed 2 h into recovery, where the growth inhibitory effect of the exercise-conditioned media was reversed. If insulin should play a role, this does not explain why growth repression is not seen in MCF-7 cells incubated with serum taken 2 h into the recovery. Glucagon and GLP-1 did not change during the exercise or recovery period.

## CONCLUSION

Physical activity is associated with significant reductions in the development of cancers. In this study, we showed that factors secreted from working muscles could inhibit cancer cell growth and induce apoptosis and that addition of anti-OSM antibodies halved this response, indicating that myokines might play a role in the protective effect of exercise on cancer.

## ACKNOWLEDGMENTS

Ruth Rousing, Hanne Villumsen, and Marianne Fregil are acknowledged for their technical assistance.

## GRANTS

The Centre of Inflammation and Metabolism is supported by a grant from the Danish National Research Foundation (no. 02-512-55). P. Hojman was supported by the Danish Medical Research Council, and the study was further supported by grants from the Augustinus Foundation, Fabrikant Einar Willumsens Foundation, and Bdr. Hartmanns Foundation.

## DISCLOSURES

The authors have no competing interests to declare.

## REFERENCES

1. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer* 9: 361–371, 2009.
2. Barnard RJ, Gonzalez J, Liva M, Ngo TH. Effects of a low-fat, high-fiber diet and exercise program on breast cancer risk factors in vivo and tumor cell growth and apoptosis in vitro. *Nutr Cancer* 55: 28–34, 2006.
3. Davis AR, Schilling E, Wellberg B, Macias W, Porter S, Riechman SE, Fluckey JD. Chemotherapeutic properties of myokines released from “E-stim” skeletal muscle. *14th International Conference Biochemistry of Exercise* (Abstract no. 30), 2009.
4. Douglas AM, Grant SL, Goss GA, Clouston DR, Sutherland RL, Begley CG. Oncostatin M induces the differentiation of breast cancer cells. *Int J Cancer* 75: 64–73, 1998.
5. Friedenreich CM, Cust AE. Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. *Br J Sports Med* 42: 636–647, 2008.
6. García-Tuñón I, Ricote M, Ruiz A, Fraile B, Paniagua R, Royuela M. OSM, LIF, its receptors, and its relationship with the malignancy in human breast carcinoma (in situ and in infiltrative). *Cancer Invest* 26: 222–229, 2008.
7. Grant SL, Begley CG. The oncostatin M signalling pathway: reversing the neoplastic phenotype? *Mol Med Today* 5: 406–412, 1999.
8. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 140: 883–899, 2010.
9. Hojman P, Brolin C, Gissel H, Brandt C, Zerahn B, Pedersen BK, Gehl J. Erythropoietin over-expression protects against diet-induced obesity in mice through increased fat oxidation in muscles. *Plos One* 4: e5894, 2009.
10. Hutt JA, DeWille JW. Oncostatin M induces growth arrest of mammary epithelium via a CCAAT/enhancer-binding protein delta-dependent pathway. *Mol Cancer Ther* 1: 601–610, 2002.
11. Jorcyk C, Holzer R, Ryan R. Oncostatin M induces cell detachment and enhances the metastatic capacity of T-47D human breast carcinoma cells. *Cytokine* 33: 323–336, 2006.
12. Liu J, Li C, Ahlborn TE, Spence MJ, Meng L, Boxer LM. The expression of p53 tumor suppressor gene in breast cancer cells is down-regulated by cytokine oncostatin M. *Cell Growth Diff* 10: 677–683, 1999.
13. Liu J, Spence MJ, Zhang YL, Jiang Y, Liu YE, Shi YE. Transcriptional suppression of synuclein gamma (SNCG) expression in human breast cancer cells by the growth inhibitory cytokine oncostatin M. *Breast Cancer Res Treat* 62: 99–107, 2000.
14. Matthews VB, Aström MB, Chan MH, Bruce CR, Krabbe KS, Prelovsek O, Akerström T, Yfanti C, Broholm C, Mortensen OH, Penkova M, Hojman P, Zankari A, Watt MJ, Bruunsgaard H, Pedersen BK, Febbraio MA. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia* 52: 1409–1418, 2009.
15. McTiernan A. Mechanisms linking physical activity with cancer. *Nat Rev Cancer* 8: 205–211, 2008.
16. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy A, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 317: 121–124, 2007.
17. Neilson HK, Friedenreich CM, Brockton NT, Millikan RC. Physical activity and postmenopausal breast cancer: proposed biologic mechanisms and areas for future research. *Cancer Epidemiol Biomarkers Prev* 18: 11–27, 2009.
18. Nicolini A, Carpi A, Rossi G. Cytokines in breast cancer. *Cytokine Growth Factor Rev* 17: 325–337, 2006.
19. Nielsen AR, Hojman P, Erikstrup C, Fischer CP, Plomgaard P, Mounier R, Mortensen OH, Broholm C, Taudorf S, Krogh-Madsen R,

- Lindegard B, Petersen AMW, Gehl J, Pedersen BK.** Association between IL-15 and obesity: IL-15 as a potential regulator of fat mass. *J Clin Endocrinol Metab* 93: 4486–4493, 2008.
20. **Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK.** Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol (Lond)* 508: 949–953, 1998.
21. **Pedersen BK.** The disease of physical inactivity—and the role of myokines in muscle—fat cross talk. *J Physiol* 587: 5559–5568, 2009.
22. **Pedersen BK, Febbraio MA.** Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 88: 1379–1406, 2008.
23. **Pollak M.** Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 8: 915–928, 2008.
24. **Rohde T, MacLean DA, Richter EA, Kiens B, Pedersen BK.** Prolonged submaximal eccentric exercise is associated with increased levels of plasma IL-6. *Am J Physiol Endocrinol Metab* 273: E85–E91, 1997.
25. **Soliman S, Aronson WJ, Barnard RJ.** Analyzing Serum-Stimulated Prostate Cancer Cell Lines After Low-Fat, High-Fiber Diet and Exercise Intervention. *Evid Based Complement Alternat Med.* In press.
26. **Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B.** Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol* 529: 237–242, 2000.
27. **Thune I, Furberg AS.** Physical activity and cancer risk: dose-response and cancer, all sites and site-specific. *Med Sci Sports Exerc* 33, 6 Suppl: S530–S550, 2001.
28. **World Cancer Research Fund and American Institute of Cancer Research.** *Food, Nutrition, Physical Activity, and the Prevention of Cancer. World Cancer Research Fund and American Institute of Cancer Research*, 2007.

