Inflammatory Biomarkers after an Exercise Intervention in Acute Lymphoblastic Leukemia Survivors

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Abstract

Background

Cancer survivors show increased risk for non-communicable diseases and chronic low-grade inflammation characterizes the development of such diseases. We investigated inflammatory plasma protein profiles of survivors of acute lymphoblastic leukemia (ALL) in comparison to healthy controls and after an intervention with a home-based exercise program.

Procedure

Survivors of ALL aged 16-30 years (n=21) with a median time of 15.9 years from diagnosis, and sex- and age-matched healthy controls (n=21), were studied. Stored plasma samples were analyzed with Olink's 92-protein-wide Inflammation panel in 21 ALL long-term survivors at baseline, after a 16-week home-based exercise intervention (n=17) and in 21 age- and sex-matched controls at baseline. Protein expression levels were compared between the groups.

Results

Inflammatory protein levels did not differ between the survivors and controls at baseline. Significantly reduced levels after the intervention were found in 11 proteins related to either vascular inflammation, insulin resistance, or both: TNFSF14, OSM, MCP-1, MCP-2, FGF-21, CCL4, TGF-alpha, TRAIL, ADA, CXCL6, and LAP TGF-beta-1.

Conclusions

The ALL survivors were not significantly more affected by inflammation than controls at baseline. The survivors’ 16-week physical exercise intervention led to significant beneficial change in inflammation protein levels. Physical exercise should be promoted for survivors of cancer.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood with a 5-year-survival rate of over 90% in many high-income countries. Unfortunately, due to ever more effective and intensive treatment protocols, the growing population of survivors tend to suffer from serious acute and late adverse effects. ALL survivors are at an increased risk for premature development of non-communicable diseases such as emerging metabolic syndrome (MetS) and cardiovascular disease (CVD). Chronic low-grade inflammation, a hallmark of aging, is widely considered to be an underlying factor in the development of non-communicable diseases. Studies on epigenetics, telomere lengths and chronic inflammation have shown that ALL survivors are in fact aging in an accelerated way. Protein expression studies, or expression proteomics, have revealed that the changes that happen with aging in one’s proteome correlate with the protein profiles in age-related diseases, such as CVD.
Besides aging, elevated levels of inflammatory proteins are also associated with excess adipose tissue and insulin resistance (IR)\textsuperscript{12,13}, which are considered catalysts in developing the full spectrum of MetS traits\textsuperscript{14}. To mitigate the inflammatory burden, even a short two-week high-intensity intermittent training program, somewhat similar to ours\textsuperscript{15}, has been shown to be effective in an overweight and obese male cohort\textsuperscript{12}.

Few studies have used extensive proteomic panels of inflammation markers to explore the process of low-grade inflammation, and even fewer have done so among ALL survivors. Hence, the aim of this study was to fill this knowledge gap by investigating the inflammatory changes that take place after ALL treatment and explore the effects of a physical activity intervention on the inflammatory protein profile of survivors of ALL.

**Results**

No statistically significant differences in the inflammatory protein concentrations at baseline between the long-term survivors of ALL and their age- and sex-matched controls were observed (Fig. 1).

However, a statistically significant change in the inflammatory protein profile of the 17 ALL survivors who completed the physical exercise intervention was observed (Fig. 2). Plasma concentrations of 11/92 analyzed proteins were significantly lower post-intervention than at baseline among ALL survivors (Fig. 3). In a descending order of uncorrected statistical significance, the 11 proteins included: TNFSF14, OSM, MCP-1, MCP-2, FGF-21, CCL4, TGF-alpha, TRAIL, ADA, CXCL6, and LAP TGF-beta-1 (Table 1). Descriptions of the functions of the 11 proteins are presented in Table 2.
Table 1
Corrected and uncorrected p-values of the 11 proteins with significantly changed expression levels after the intervention.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Corrected p-value</th>
<th>Uncorrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFSF14/LIGHT</td>
<td>0.0069</td>
<td>0.000169</td>
</tr>
<tr>
<td>OSM</td>
<td>0.0069</td>
<td>0.000209</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>0.0069</td>
<td>0.000278</td>
</tr>
<tr>
<td>MCP-2/CCL8</td>
<td>0.0069</td>
<td>0.000322</td>
</tr>
<tr>
<td>FGF-21</td>
<td>0.0069</td>
<td>0.000375</td>
</tr>
<tr>
<td>CCL4/MIP-1-beta</td>
<td>0.0093</td>
<td>0.000608</td>
</tr>
<tr>
<td>TGF-alpha</td>
<td>0.0202</td>
<td>0.0018</td>
</tr>
<tr>
<td>TRAIL/TNFSF10</td>
<td>0.0202</td>
<td>0.00182</td>
</tr>
<tr>
<td>ADA</td>
<td>0.0202</td>
<td>0.00198</td>
</tr>
<tr>
<td>CXCL6</td>
<td>0.0227</td>
<td>0.00247</td>
</tr>
<tr>
<td>LAP TGF-beta-1</td>
<td>0.0290</td>
<td>0.00347</td>
</tr>
</tbody>
</table>
Table 2

Descriptions of the functions of the 11 proteins with significantly lowered expression levels, presented in a descending order of uncorrected statistical significance.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFSF14/LIGHT</td>
<td>Pro-inflammatory cytokine important in stimulating entry of T-cells into inflamed tissues and in induction of matrix metalloproteinases in macrophages, which has been suggested to induce atherosclerosis development. Reported to be involved in endothelial inflammation by potently inducing inflammatory responses in endothelial cells. In T2DM patients, LIGHT plasma levels have been reported to be increased, which suggests a role in T2DM progression by its attenuating effect on insulin secretion in pancreatic islet cells alongside the endothelial inflammation.</td>
</tr>
<tr>
<td></td>
<td><strong>Role in:</strong> Vascular inflammation, insulin metabolism.</td>
</tr>
<tr>
<td>OSM</td>
<td>Cytokine playing an important role at least in regulating the production of cytokines, such as IL-6, G-CSF, and GM-CSF in endothelial cells in inflammation. Described as both pro- and anti-inflammatory. Despite promising results in animal models concerning, e.g., MetS, ameliorating IR and lipid accumulation or thrombus reduction, the knowledge of the functions of OSM in humans is still insufficient.</td>
</tr>
<tr>
<td></td>
<td><strong>Role in:</strong> Most likely vascular inflammation.</td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>Chemokine related to atherosclerosis and endothelial cells as it is anchored to their cell membranes, in addition to being secreted by various immune cells. Its activation occurs when cleaved by MMP-12, and upon binding to its receptors, CCR2 and CCR4, it provokes movement of monocytes and basophils to inflammatory sites, such as arterial walls, by chemotaxis. There are reports of significantly higher levels of MCP-1 in obese insulin-resistant adults.</td>
</tr>
<tr>
<td></td>
<td><strong>Role in:</strong> Vascular inflammation, IR.</td>
</tr>
<tr>
<td>MCP-2/CCL8</td>
<td>Attracts immune cells to inflamed tissues by chemotaxis as the chemokine superfamily members do. On the other hand, it can also inhibit the chemotactic activity of MCP-1. MMP-12 has been shown to cleave MCP-2 resulting in its inactivation.</td>
</tr>
<tr>
<td></td>
<td><strong>Role in:</strong> Most likely vascular inflammation.</td>
</tr>
<tr>
<td>FGF-21</td>
<td>Has the ability to stimulate glucose uptake in differentiated adipocytes via the expression of glucose transporter SLC2A1/GLUT1. It is a cytokine with anti-inflammatory effects and a relation to IR. Elevated levels suggest resistance to FGF-21. Even involvement in beta-cell failure has been suggested.</td>
</tr>
<tr>
<td></td>
<td><strong>Role in:</strong> IR.</td>
</tr>
<tr>
<td>CCL4/MIP-1β</td>
<td>Type of CC chemokine with inflammatory properties by chemotaxis. Binds to CCR5, which has been reported to clearly have a role in human CVD. However, CCL4 is not the main ligand for CCR5 in the pathogenesis of atherosclerosis, but it has been reported to likely have an important role in attracting inflammatory cells into plaques, and to be associated with coronary atherosclerosis, as well as being present in human atherosclerotic plaque.</td>
</tr>
<tr>
<td></td>
<td><strong>Role in:</strong> IR.</td>
</tr>
<tr>
<td>Protein</td>
<td>Function</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Role in:</strong></td>
<td>Vascular inflammation.</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Cytokine that induces epithelial development as it binds to its receptor EGFR. Its involvement has been described in some inflammatory diseases and several solid tumors, but seemingly not in CVD or MetS.</td>
</tr>
<tr>
<td><strong>Role in:</strong></td>
<td>Most likely vascular inflammation.</td>
</tr>
<tr>
<td>TRAIL/TNFSF10</td>
<td>Involved in vasodilatation, atherosclerosis and other inflammation-related diseases. Protective role in DM suggested due to findings in T2DM animal models.</td>
</tr>
<tr>
<td>ADA</td>
<td>Important enzyme in purine metabolism and breakdown of adenosine into nucleic acids. Binds to its receptor DPP4. Purine metabolism's previous stages include turnovers of ATP and ADP, which both trigger negative events like inflammation in blood vessels in their extracellular forms. These turnovers are catalyzed by the enzymes CD39 and CD73, respectively, resulting in adenosine. These enzymes are expressed by endothelial cells and ADA is present even on the extracellular surface of endothelial cells of small coronary arteries, in addition to being present in erythrocytes. Adenosine has wide effects in humans such as in glucose uptake, anti-inflammatory effects, vasodilation, or atrioventricular block when administered therapeutically. Adenosine even reduces T-cell proliferation and cytokine production, which is counteracted by ADA and DPP4 together. Additionally, DPP4 inhibitors are established anti-diabetics.</td>
</tr>
<tr>
<td><strong>Role in:</strong></td>
<td>Vascular inflammation, IR.</td>
</tr>
<tr>
<td>CXCL6</td>
<td>CXC cytokine subfamily member. Binds to CXCR1 and CXCR2, both found on endothelial cells, but only the latter is involved in ligand-mediated angiogenesis. CXCR2 antagonists have been proposed as potential drug targets to alleviate atherogenesis, but studies on endothelial dysfunction and CXCL6 do not exist.</td>
</tr>
<tr>
<td><strong>Role in:</strong></td>
<td>Most likely vascular inflammation.</td>
</tr>
<tr>
<td>LAP TGF-β1</td>
<td>LAP, the regulatory protein of TGF-β1, is the active protein of the complex. The regulatory activity of TGF-β is adjusted by the presence of inflammatory cytokines, costimulatory molecules, and state of differentiation of the cell affected. Moreover, inflammatory processes are regulated with regards to their activation and resolution. Altogether, TGF-β inhibits the development of pathological autoimmunity. TGF-β has even been suggested to regulate atherogenesis in humans as it is reported to have anti-atherosclerotic properties in animals.</td>
</tr>
<tr>
<td><strong>Role in:</strong></td>
<td>Vascular inflammation.</td>
</tr>
<tr>
<td>Protein</td>
<td>Function</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Abbreviations: ADA, adenosine deaminase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; BMI, body mass index; CCL, chemokine (C-C motif) ligand; CCR, C-C chemokine receptor; CSF, colony stimulating factor; CVD, cardiovascular disease; CX3CL1, chemokine (C-X3-C motif) ligand 1; CXC, C-X-C motif; CXCL, chemokine (C-X-C motif) ligand; CXCR, CXC chemokine receptor; DM, diabetes mellitus; DPP4, dipeptidyl peptidase-4; EGFR, epidermal growth factor receptor; FGF-21, fibroblast growth factor 21; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IL-6, interleukin-6; IR, insulin resistance; LAP TGF-β1, latency associated peptide transforming growth factor beta; MCP, monocyte chemotactic protein; MetS, metabolic syndrome; MIP-1β, macrophage inflammatory protein-1-beta; MMP, matrix metalloproteinase; OSM, Oncostatin-M; T2DM, type 2 diabetes mellitus; TGF-α, transforming growth factor alpha; TGF-β, transforming growth factor beta; TNFSF14/LIGHT, tumor necrosis factor superfamily member 14; TRAIL/TNFSF10, tumor necrosis factor-related apoptosis-inducing ligand 10</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

ALL survivors did not differ in their plasma inflammatory protein levels compared to their age- and sex-matched controls. This was unexpected in light of reports of ALL survivors being at risk for inflammation and accelerated aging and considering the diseases affiliated with these conditions, such as MetS. Contrarily, this is one of the first reports of such a result when using an inflammatory protein panel of this size. This finding could be explained by the fact that the original study found no significant differences in, e.g., BMI, systolic blood pressure, fasting glucose, high-density lipoprotein cholesterol, or triglycerides between the survivors and controls at baseline. This may also explain why no signs of accelerated aging in the form of disease-like protein profiles were found in this cohort of ALL survivors compared to controls, despite having cardiovascular risk factors as described in the original study. In the original study, the ALL survivor cohort was found to be similar to unparticipating survivors in leukemia-related factors and regarding, e.g., BMI and level of physical activity, which undermines the possibility of attributing this result to an unrepresentative sample. The ALL survivors were adolescents and young adults (ages 16-30 years) at the time of the study, so the lack of differences in protein expression levels at baseline compared to healthy controls may be due to the long duration of time it takes to develop a pathological disease state, such as MetS or CVD. Furthermore, the control group included 7/21 overweight (BMI>25) subjects and the patient group 8/21 subjects, which likely narrows their proteomic differences. Ten of the 21 controls had a below-average physical condition compared to 16/21 in the patient group, when physical condition was measured as peak oxygen uptake (VO2 peak) in proportion to weight and classified by age and gender reference values. Additionally, a large inter-individual variation in protein profiles compared to rather stable intra-individual levels may have led to a lack of power in the analysis between the survivors and the controls.

We found a decrease in 11 proteins related to either vascular inflammation, insulin resistance, or both after the intervention. In the original study, endothelial dysfunction and IR were significantly ameliorated after the intervention.
TNFSF14/LIGHT decreased most significantly during the exercise intervention compared to baseline. This protein has been found to be involved in endothelial inflammation and impaired insulin secretion. The ALL survivors had a significant improvement in their endothelial function as measured by the left common carotid artery intima media thickness (IMT) and flow mediated dilation (FMD) of the left brachial artery in the original study. Our findings support the perception of LIGHT being involved in endothelial inflammation and atherogenesis, and that increased physical activity may decrease these vascular pathologies. The survivors’ insulin metabolism, i.e. IR, improved during the intervention and the decrease in LIGHT expression levels is in line with the observed improved metabolism.

The function of OSM is not well defined. Regarding its pro- versus anti-inflammatory effects, more documentation of the pro-inflammatory effects exists in humans, at least when it comes to vascular injury. Endothelial cells express high levels of OSM receptor, making them one of the primary target cells for OSM, which is suggested to have an indirect ability to increase vascular permeability and perivascular infiltration of immune cells at the sites of tissue damage. The decrease in OSM after the intervention further supports its role as pro-inflammatory in endothelial inflammation.

MCP-1 plays a role in endothelial dysfunction caused by inflammation, and significantly elevated levels have been reported in obese insulin-resistant adults. The decrease in MCP-1 is in agreement with the clinical findings of the original study in both these regards. MCP-2/CCL8 is known to inhibit the chemotactic activity of MCP-1, whose levels decreased during the intervention. Thus, we suggest that the lower levels of MCP-2 after the intervention, might be related to the lower need to inhibit MCP-1, which has been described to have a firmer link to endothelial inflammation than MCP-2. Another suggestion in which MCP-2 could be associated with endothelial dysfunction, includes the fact that matrix metalloproteinase MMP-12, which cleaves MCP-1, has also been shown to cleave MCP-2/CCL8 at a position which inactivates MCP-2, causing the truncated form to serve as a receptor antagonist suppressing recruitment of macrophages. If there was a reduced level of MCP-1 to be cleaved by MMP-12 in our survivors after the intervention, perhaps more MCP-2 was cleaved and inactivated, leading to attenuated inflammation.

We found support for the survivors’ improved insulin metabolism even through FGF-21, whose plasma expression levels decreased during the intervention. This cytokine is related to IR and MetS even in pediatric populations with elevated levels suggesting resistance to it. Even involvement in beta-cell failure has been suggested. Due to the cytokine’s stimulatory role in glucose uptake, there have been promising findings in the form of dyslipidemia improving outcomes from several of the clinical trials that are developing long-acting FGF-21 analogs. Altogether, the literature indicates that FGF-21 in humans is an insulin-dependent hormone with primarily postprandial release in addition to exercise-induced release from mainly the liver. In keeping with our MCP-1 data and the original study’s significantly reduced IR after the intervention, the exercise program reduced also the levels of FGF-21, which is in alignment with a reported positive correlation between plasma insulin and FGF-21 levels.
The recurring association between a marker of vascular inflammation (Table 2) and the improvement in our survivors’ endothelial function was seen in the case of CCL4 too, as its levels decreased from baseline to post-intervention. A study on children with untreated primary hypertension also discovered CCL4/MIP-1-beta to be significantly elevated when compared to healthy peers. Furthermore, patients with MetS have also been reported to have significantly elevated levels of CCL4 and its receptor CCR5. The same study reported both CCL4 and CCR5 levels to significantly reduce in response to a low-dose statin treatment, of which the former’s reduction has been reported by others as well, albeit in a cohort of patients with coronary artery disease.

It might be that TGF-alpha has no direct association to CVD (Table 2), but the significantly lowered levels of the protein in our study from baseline to post-intervention, could be explained indirectly via its effects on matrix metalloproteinases such as MMP-1. This in turn has been described to be associated with arterial stiffening in type 1 diabetes mellitus patients and elevated in T2DM patients. However, our analysis also included MMP-1 and no significant change was seen in its expression levels, which suggests that the change in TGF-alpha’s level might also have been due to its capability to affect angiogenesis or a novel description of TGF-alpha’s relation to endothelial dysfunction.

Our survivors had also lower plasma expression levels of TRAIL after the intervention than at baseline. TRAIL has previously been suggested to have a protective role in endothelial dysfunction and in ischemic vascular diseases based on clinical evidence. On the contrary, we report an opposite association when it comes to endothelial dysfunction and TRAIL. It has even been suggested as protective in diabetes mellitus. It could be that the exercise program was sufficient enough to improve the endothelial function, thereby reducing the need for the probable antioxidant effect of TRAIL that has recently been suggested to lie behind its vasoprotective effects. Alternatively, the vascular health of the survivors could have been sufficient to not require TRAIL’s atherosclerotic plaque-stabilizing protection.

We saw as well a significant reduction in the levels of ADA from baseline to post-intervention, which is in line with the significant evidence of adenosine being involved in vascular barrier dysfunctions and endothelial dysfunction (Table 2). How to affect adenosine pharmacologically in the context of CVD has been previously discussed and clopidogrel is already an established example of a drug affecting the purinergic metabolism. Instead, we exhibit evidence of our 16-week exercise program affecting this metabolism in the form of lowered levels of ADA without any medication. Additionally, receptor inhibitors of ADA, DPP4 inhibitors, are established anti-diabetics.

No studies on endothelial dysfunction and CXCL6 seem available in the literature, but our results showing significantly-reduced levels of CXCL6 from baseline to post-intervention suggest it may have a role as a biomarker of endothelial inflammation, as the endothelial function was improved at the post-intervention phase.

TGF-beta has been suggested to regulate atherogenesis even in humans, and in animals it is reported to have anti-atherosclerotic properties, mainly through inhibition of T-cells with atherosclerotic functions, but
possibly even by directly regulating endothelial cells and macrophages among other cell types. We found the plasma LAP TGF-beta-1 expression levels to be significantly lower after the exercise program than before it, which indicates an increase in the active form (TGF-beta-1) as the latent form (Table 2) reduced. Thus, we suggest TGF-beta to be active in resolving endothelial inflammation. The active form was not measured in this study.

In summary, the long-term survivors of ALL did not have a difference in their inflammatory burden compared to their peers in our cohort of 21 former patients and 21 healthy controls. Instead, a home-based 16-week exercise intervention resulted in reduced expression profiles of low-grade inflammation biomarkers in the plasma of 17 ALL survivors who completed the program. The eleven proteins that were significantly decreased in this study are involved most often in vascular inflammation, like in the case of TNFSF14, MCP-1, CCL4, TRAIL, ADA, and LAP TGF-beta-1. Some of the identified proteins do not have a clear role in endothelial inflammation, but we consider OSM, MCP-2, TGF-alpha, and CXCL6 to have an association with endothelial dysfunction when the proteomic results are considered together with our clinical data. Some of the proteins were found to be mostly or additionally related to IR, like in the case of MCP-1, FGF-21, TRAIL, and ADA, or insulin metabolism in the case of TNFSF14/LIGHT. Although the roles of some of the identified proteins were harder to interpret based on available data, the observed changes in their plasma expression levels after the intervention reflect a healthier inflammatory profile, with the exception of TRAIL, whose previously described protective role we now question.

Despite the fact that no change was observed in some proteins related to endothelial inflammation (such as fractalkine/CX3CL1, CSF-1, or HGF) following completion of the intervention, we can state that a home-based exercise program does alleviate cardiovascular pathology on a biomarker level in a population with similar baseline inflammatory profiles as its healthy peers. We consider there to be room to increase the amount of these types of exploratory studies amongst ALL survivors as the population is at risk for premature morbidity and can benefit from early detection of localized pathologies which precede systemic effects.

Limitations of this study were the relatively small sample size and drop-out of four subjects before completion of the intervention. Furthermore, five of the subjects had received cranial irradiation, leading to a heterogeneity of the survivor group. Another weakness was the lack of follow-up samples from controls.

Future studies should involve larger sample sizes for greater statistical power and additional interventions to reduce the burden of low-grade inflammation on these young adults. Studies with more recently treated patients should also be performed to better evaluate the potential consequences of today’s more intensive chemotherapy-only treatment on ALL survivors’ inflammatory profiles compared to their healthy peers. The improvements seen in inflammatory protein profiles after our exercise intervention further emphasize the role of exercise in mitigating the inflammatory state and risk of CVD among survivors of ALL. We would like to suggest that these kind of positive effects following physical
activity could be achieved by the general public as well, as the survivors’ inflammatory profiles did not differ from their controls’ profiles at baseline.

Methods

This was an add-on study of a non-randomized controlled intervention cohort study\textsuperscript{15,22}, which had a cross-sectional first phase with controls, and a second phase consisting of an exercise intervention for the ALL survivors. Stored plasma samples from the original study in Turku, Finland, that compared the cardiac and endothelial health in a cohort of ALL survivors and controls in 2007-2008, were used in this study. This add-on study falls under the original study's ethical permission from the Commission on Ethics of Southwest Finland Hospital District (reference number 45/2007) and was performed in accordance with the Declaration of Helsinki. Written informed consent from each participant was obtained as part of the original study.

Study population and the exercise intervention

The study population and the intervention have been previously described\textsuperscript{15,20}. In brief, the population consisted of 21 (11 females) long-term survivors of ALL aged 16-30 years, diagnosed with ALL between 1986–1996 and treated according to the NOPHO ALL86 or a later NOPHO protocol. Healthy control subjects consisted of 21 (11 females) age- and sex-matched siblings (5/21), friends (11/21), or other non-athletic adolescents and adults (5/21). At baseline, 21 ALL survivors and 21 age- and sex-matched controls underwent a physical examination and provided blood samples. Seventeen of 21 survivors completed a home-based exercise intervention\textsuperscript{22} of 16 weeks in mean (4/21 survivors dropped out before starting the intervention). The same parameters as at baseline were collected for these 17 ALL survivors after completion of the exercise intervention.

The exercise program was developed by experts in sports and exercise medicine and exercise science. In brief, the subjects were provided illustrated instructions for the home-based muscle-training program and told to perform it 3-4 times per week. They were also encouraged to do 30-minute aerobic exercise sessions at least 3 times per week either as a warm-up or separate to the muscle training.

Proximity extension assay, PEA

In total, 59 plasma samples stored in Turku and collected in 2007-2008 (n=21 ALL survivors at baseline, n=17 ALL survivors after the intervention, and n=21 controls at baseline) were analyzed for 92 proteins related to inflammation using the Olink Target 96 Inflammation panel (article number 95302, Olink Proteomics, Uppsala, Sweden) at the Clinical Biomarkers Facility at SciLifeLab (Uppsala, Sweden). The samples were thawed, centrifuged, measured and refrozen to -80°C for shipment from Turku to Uppsala according to the company’s instructions. The samples were transferred to a 96-well PCR (polymerase chain reaction) plate such that they were both randomized and de-identified. All 59 samples passed quality control.
The concentrations of the proteins analyzed with the Inflammation panel are reported using a unit of relative quantification, which means that the values cannot be compared between different proteins, only between different samples. This unit, called Normalized Protein eXpression (NPX), is an arbitrary unit defined by Olink to achieve minimal intra- and inter-assay variation. NPX is on a log2 scale.\textsuperscript{44}

**Statistical analysis**

Inflammation panel data in NPX values were analyzed using two paired t-tests, Benjamini-Hochberg method for p-value correction with 5% false discovery rate, and a distribution boxplot (Supplementary Figure S1) for outlier analysis. P-values <0.05 were considered statistically significant after correction with the Benjamini-Hochberg method. A principal component analysis (PCA) plot (Supplementary Figure S2) assessed possible outlier samples. No outliers were found, and all 59 samples could be included in the paired t-tests, which were performed to compare results of the ALL survivors at baseline versus after intervention, and ALL survivors at baseline versus controls. Only the 17 survivors who completed the assessment both before and after the intervention were included in the paired t-test between them. These analyses were performed using R version 3.6.1. for Windows.\textsuperscript{45} For interpretation of results, clinical data from the original study were utilized.

**Declarations**

**Data Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Author Contributions**

L.J., H.N., T.L., and A.H.S. designed the research study. L.J. handled the samples. T.L. and L.J. gathered data. T.L. analyzed the data and wrote the draft of the manuscript. L.J., A.H., H.N., and A.H.S revised the manuscript. All authors reviewed the manuscript critically and approved the final version of it.

**Conflict of Interest Statement**

The authors declare no competing interests.

**References**


44. What is NPX? Olink https://www.olink.com/question/what-is-npx/.


**Figures**
Figure 1

Volcano plot of the paired t-test between the ALL survivors at baseline and the controls. No statistically significant differences in protein expression levels were found after correction. The dotted line represents the uncorrected significance threshold of 0.05. On the y-axis are p-values and on the x-axis is the log of the fold change between the two groups.
Figure 2

Volcano plot of the paired t-test between the ALL survivors at baseline and after intervention. The proteins with statistically significant changes in expression level between the two time points are labeled with names. The dotted line represents the uncorrected significance threshold of 0.05. On the y-axis are p-values and on the x-axis is the log of the fold change between the two groups.
Figure 3

Boxplots of the 6 proteins with most significant changes in protein expression levels (a) and of the other 5 proteins with statistically significant changes in protein expression levels (b) following paired t-tests between p1 (ALL survivors at baseline) and p2 (ALL survivors after intervention). NPX (Normalized Protein eXpression) is the unit on the y-axis and colors denote sample type (p1 or p2). Protein names and their Olink identification numbers are displayed above the boxplots.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 3.1SupplementalFigureS1DistributionboxplotWITHlegend.png
- 3.2SupplementalFigureS2PCAplobWITHlegend.png