Lifestyle Behaviors and Inflammatory Markers in Childhood Cancer Survivors: a systematic review

Writing Assignment Biology of Disease

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1 Abbreviations

ALL: Acute Lymphoblastic Leukemia

AML: Acute Myelogenous Leukemia

BMI: Body Mass Index

CAJ: Clear Apple Juice

CAYA: Childhood, adolescent, and young adult

CI: Confidence Interval

CML: Chronic Myelogenous Leukemia

CNS: Central Nervous System

DXA: Dual-Energy X-Ray Absorptiometry

GVHD: Graft-versus-host disease

HCT: Hematopoietic Cell Transplantation

Hs-CRP: High Sensitivity C-reactive protein

IL: Interleukin

IQR: Interquartile Range

LBM: Lean Body Mass

MPO: Myeloperoxidase

NR: Not Reported

PFM: Percent Fat Mass

PGJ: Purple Grape Juice

RCT: Randomized Controlled Trial

ROS: Reactive Oxygen Species

SD: Standard Deviation

TBI: Total Body Irradiation

TNF: Tumor Necrosis Factor

2 Abstract

Background: Survivors of childhood-, adolescent- and young adult (CAYA) cancers often face comorbidities such as cardiovascular disease and osteoporosis earlier than expected for their age. These observed effects have previously been linked to a cancer treatment-induced pro-inflammatory state. As these late effects can greatly impact mortality and quality of life, it is important to identify factors or interventions that can delay the onset of these diseases. Previous research has shown that a poor lifestyle is a modifiable risk factor for inflammation. Consequently, healthy lifestyle choices might counteract the inflammatory processes that underly the late effects in survivors.

Aim: The aim of this systematic review was to give an overview of the evidence for the relationship between lifestyle behaviors and inflammatory markers in CAYA cancer survivors.

Methods: For this systematic review, the database PubMed was searched for articles reporting lifestyle behaviors and inflammatory markers (acute phase proteins) in CAYA cancer survivors (0-25 years at diagnosis). Lifestyle behaviors were defined as dietary intake, physical activity, vitamin D levels or intake, alcohol intake, smoking and stress (therapy). Titles and abstracts were screened by two independent reviewers and eligible studies were selected for full-text review. From all included study, we extracted data concerning study methods, characteristics of participants and outcomes along with risk of bias.

Results: In total, 722 studies were screened of which two studies were included in this review. The first study by Blair and colleagues was a randomized cross-over trial, investigating the effect of 4-week purple grape juice (pGJ) supplementation compared to 4-week clear apple juice (cAJ) supplementation on plasma inflammatory markers Myeloperoxidase (MPO) and high sensitivity C-reactive protein (hs-CRP) in survivors of various childhood cancers. There was no significant beneficial effect of pGJ- over cAJ supplementation on MPO ($p=0.15$) or hs-CRP ($p=0.37$) levels.

The second study by Ketterl and colleagues had an observational cross-sectional design, investigating the inflammatory markers Interleukin (IL)-6 and tumor necrosis factor (TNF)-α, and body composition measures (percent fat mass (PFM) and total lean body mass (LBM)) in survivors of hematopoietic cell transplantation (HCT) compared to age- and sex-matched siblings. HCT survivors had significantly higher IL-6 levels ($p<0.05$) and approximately 5% higher PFM ($p<0.005$) compared to their siblings, irrespective of previous cancer treatment. Additionally, LBM was significantly lower in HCT survivors who had received Total Body Irradiation (TBI) ($p<0.001$).

Discussion & Conclusion: The included studies suggested that although pGJ supplementation does not change inflammatory markers, low lean body mass or increased PFM might be associated with chronic inflammation and might therefore be a target for delaying late effects in childhood cancer survivors. In adult cancer survivors, lifestyle has already shown beneficial effects in inflammation and chronic diseases, which highlights the need for randomized trials investigating the relation between lifestyle, inflammation, and late effects in childhood cancer survivors.

3 Layman's Summary

This review gives an overview of all studies that have investigated the effect of lifestyle on inflammation in childhood cancer survivors from 2012 onwards. As cancer treatments continue to improve, there are more and more CAYA cancer survivors. However, these survivors often experience symptoms that are normally only expected in older adults, at an early stage. Examples of these late effects are cardiovascular disease, diabetes, and osteopenia.

The late effects in childhood cancer survivors are most likely due to cancer treatments and have been suggested to be mediated by inflammatory processes. Previous research has shown that inflammation can be caused by a poor lifestyle, such as an unhealthy diet and physical inactivity. Consequently, a healthy lifestyle might have the potential to reverse the treatment-induced inflammation and thus potentially the late effects seen in CAYA cancer survivors. Therefore, the aim of this review was to investigate whether there is a link between inflammation and lifestyle in CAYA cancer survivors.

For this aim, we searched the database PubMed and only included the articles with CAYA cancer survivors (up to 25 years at diagnosis) in which lifestyle behaviors and inflammatory markers were reported. For lifestyle, we looked specifically at dietary intake, physical activity, vitamin D intake, alcohol intake, smoking and stress (therapy). There were no restrictions for the type of cancer or age at participation in the study.

In total, 722 articles were screened and two of these studies were included in the review. One of these studies was a randomized controlled trial in 24 childhood cancer survivors, in which inflammatory markers (MPO and hs-CRP) were measured after consumption of pGJ compared to cAJ. This study was set up because flavonoids, which are contained in pGJ but not cAJ, have shown anti-inflammatory effects. The study had a cross-over design, with a run-in period where no juice was consumed, a 4-week period where some drank pGJ and others cAJ, a wash-out period with no juice consumption, and lastly a 4-week period with the other juice. No significant differences in MPO or hs-CRP were observed between the juice groups.

The other study was in childhood blood cancer survivors who had received HCT. The aim of the study was to analyze whether body composition, meaning BMI, fat mass and lean mass, and inflammatory markers (IL-6 and TNF-α) were different in HCT survivors compared to their siblings. The HCT survivors were categorized based on their cancer treatment in the past, which could either be TBI with or without brain radiation or only chemotherapy. The researchers reported that HCT survivors in all treatment groups had similar BMI as their siblings, but they had more fat mass and less lean mass, along with higher IL-6 levels.

This review shows that we only have very limited evidence of a potentially beneficial role in childhood cancer survivors regarding inflammation and chronic diseases, which highlights the need for further research.

4 Introduction

In recent years, the number of CAYA cancer survivors has increased to approximately 80% according to European cancer registries. Specifically survival of acute lymphoblastic leukemia (ALL) and non-Hodgins lymphoma has improved from 50%-85% and 74%-82%, respectively (1).
However, in the long term, CAYA cancer survivors are at risk of developing age-related health conditions at a young or middle-aged stage in life. These conditions include cardiovascular diseases, such as heart failure and coronary artery diseases, osteopenia and diabetes which often lead to frailty, disability and early mortality (2–6).

Although the pathophysiology is still mostly unknown, studies suggest that chronic, low-grade inflammation plays an important role in the accelerated aging in CAYA cancer survivors (7,8). This chronic inflammatory state is likely caused by anti-cancer treatments such as chemotherapy, radiotherapy and stem-cell transplantations, which result in accumulation of ROS (reactive oxygen species), senescent cells and DNA mutations, as well as immune activation and a pro-inflammatory phenotype (8–11). To illustrate, previous studies in survivors of ALL, (non-) Hodgkin's lymphoma and other solid tumors reported higher levels of inflammatory markers, such as CRP, IL-2, IL-10 and IL-17a, compared to controls. These inflammatory markers, in turn, stimulate production of other pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (8,9).

Recent research in middle-to older age adults has shown, that a healthy lifestyle might protect against chronic low-grade inflammation, for which several mechanisms have been proposed (12). For example, regular exercise has been associated with increased production of anti-inflammatory cytokines in skeletal muscle along with decreased production of the pro-inflammatory cytokines TNF-α and IL-6 through reduction of adipose tissue (13–16). Similarly, intake of dietary components such as fiber, whole-grains and anti-oxidant vitamins has shown potential in lowering inflammatory markers (17,18). Lastly, smoking and alcohol abuse have been linked to immune cell recruitment and liver damage, respectively, both leading to pro-inflammatory cytokine production (13).

Although a healthy lifestyle and reduced inflammation have been linked in adult cancer survivors (19–23), little is known about the interplay between these factors in CAYA cancer survivors. Therefore, a systematic review was conducted to give an overview of the current knowledge on the association between lifestyle behaviors, including physical activity, dietary intake and nutritional deficiencies, vitamin D intake and serum levels, alcohol intake, smoking and stress, and inflammatory markers as outcome in CAYA cancer survivors. It was hypothesized that healthy lifestyle behaviors are associated with lower levels of inflammatory markers.

5 Methods

Inclusion criteria

For the systematic review, all English original studies published in 2012 or later were included except (systematic) reviews, meta-analyses, and case-reports/series. Concerning participants, all studies performed in CAYA cancer survivors were included in this systematic review, defined as being diagnosed with cancer up to the age of 25 years and having completed cancer treatment prior to participation in the study. There were no restrictions for the type of cancer and the age at participation in the studies. In case of mixed samples, we only included the studies where subpopulations meeting the inclusion criteria could be separated from the total sample.

The lifestyle behaviors that were within the scope of this review were physical activity, dietary intake, smoking, alcohol intake, vitamin D intake and serum levels, and stress. For the outcome inflammation, we included studies that reported specific inflammatory markers, such as CRP, TNF-α and Interleukins.

Study selection

We identified studies by using a systematic search in PubMed (Supplemental Table 9.1). Studies that were excluded based on the age of the participants were bundled and searched for relevant information. In the title and abstract screening phase, all studies identified through PubMed were screened by one reviewer, and two independent reviewers each screened half of the identified title and abstracts. Screening was performed using the web-application Rayyan (24). If there were unsolvable discrepancies between the results of the mentioned reviewers, a third reviewer was consulted. Studies were included for a full paper review if the studies met the inclusion criteria or if it was unclear whether the article met the inclusion criteria. If there was overlap between populations in different publications, the most recent study was used, except if older publications provided more detailed information.

Data extraction and risk of bias

The extraction of data from the included articles was performed by one reviewer and checked by another reviewer. In case of conflicts that could not be resolved with discussion, a third reviewer was consulted. Data were extracted on methods (study design, number of participants, type of lifestyle behavior), characteristics of participants (age at primary diagnosis, attained age, type of cancer, type of cancer treatment, time after completion of treatment) and outcome (reported inflammatory markers). In this systematic review, only descriptive data were reported instead of statistical pooling as the studies varied in design, lifestyle behaviors and inflammatory markers.

One reviewer evaluated the risk of bias of the included studies, and it was checked by another independent reviewer. The criteria for risk of bias were based on previously described guidelines in the Cochrane Handbook for Systematic Review of Interventions and the IGHG Handbook For Guideline development (25–27) Definitions for the risk of bias criteria for both observational and randomized studies can be found in Supplemental Tables 9.2 and 9.3, respectively. In short, observational studies were assessed on selection bias (random sample with respect to cancer type, treatment, age, and history of inflammatory disease), attrition bias (loss to follow-up), detection bias (blinding of assessors) and risk of confounding (important prognostic factors considered). Randomized trials were assessed on selection bias (sequence allocation), attrition bias (loss to follow-up) and blinding procedures (detection-and performance bias).

6 Results

In total, 722 articles were retrieved using the PubMed search strategy (Supplemental Table 9.1) and all articles were screened for eligibility by two independent researchers. Of the 722 studies, 719 were excluded based on study population (e.g. elderly, current patients) or a lack of reported lifestyle behaviors or inflammatory markers. In total, three studies were selected for full-text review, of which two fulfilled the inclusion criteria and were included. The third study was excluded after discussion based on the type of intervention, which was psychosocial therapy rather than lifestyle behavior change. The selection process is shown in **Figure 1**.

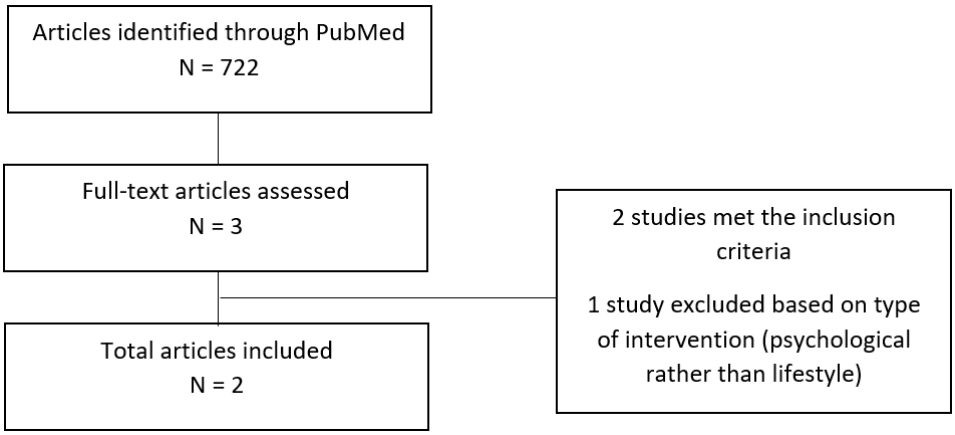

***Figure 1**. Overview of selection process.*

The first study was a randomized cross-over trial performed by Blair and colleagues, investigating the effect of pGJ on inflammatory markers (28), and the other study by Ketterl and colleagues had an observational design, investigating body composition and inflammatory markers in HCT survivors, categorized in three treatment groups, compared to age-and sex-matched siblings (29) (**Table 1**). The results of both studies are summarized in **Table 2** and Supplementary Table 9.4.

***Table 1.** Characteristics of included studies*

First author, year	Country, city	Study design	Number of participants (case/ controls)	Age diagnosis (years)	Attained age (years) (SD)	Type of cancer
Blair C, 2014	USA, Minneapolis	RCT cross-over	24 (12/12) *	3.6 (range 1.5-6.1)	16.4 (range 13.7-17.2)	All types
Ketterl TG, 2018	USA, Seattle	Observational study	151/92 **	NR	HCT survivors (Three treatment groups): 24 (6) Siblings: 24 (0.7)	Hematologi-cal cancers (HCT survivors)

**Both cases and controls were cancer survivors, randomly assigned to the intervention or control group. ** Cases were 151 HCT survivors and 92 of their siblings were defined as controls. HCT survivors were categorized in three groups based on previous cancer treatment, being TBI with CNS radiation, TBI without CNS radiation or chemotherapy only. Abbreviations: HCT= Hematopoietic Cell Transplantation; NR=Not Reported; RCT= Randomized Controlled Trial; SD=Standard Deviation*.

Purple grape juice supplementation and inflammation in childhood cancer survivors

As shown in **Table 2**, Blair and colleagues (28) performed an RCT with a cross-over design in 24 childhood cancer survivors (off-treatment for three years), studying the effect of pGJ supplementation on vascular health and inflammation compared to cAJ. Participants drank 6 ounces of pGJ and cAJ two times a day for 4 weeks, separated by 4-week wash-out periods. Plasma samples for biomarker measurements were collected after the 4-week run-in period and after each supplementation period. Participants were either survivor of hematopoietic malignancies (n=12), mostly of ALL, or of solid tumors (n=12), such as Central nervous system (CNS) or bone tumors. There was no significant beneficial effect of pGJ as compared to cAJ on plasma inflammatory markers MPO [adjusted difference pGJ and cAJ 0.92 ng/ml (95% CI 0.82-1.03), p=0.15] or hs-CRP [adjusted difference pGJ and cAJ 1.34 mg/l (95% CI 0.69-2.62), p=0.37]. There was no evidence for a period effect, carryover effect or compliance difference in both juice groups.

Body composition and inflammation in HCT survivors

Ketterl and colleagues aimed to study differences in body composition and inflammatory markers between 151 HCT survivors, who were at least two years after completion of treatment for a hematological malignancy, and their age- and sex-matched siblings (**Table 2**) (29). HCT survivors were categorized into three groups based on previous cancer treatment, being ① TBI + CNS radiation (n=31), ② TBI without CNS radiation (n=85), and ③ chemotherapy (n=35)**.** Fat mass and lean body mass were assessed with Dual-Energy X-Ray Absorptiometry (DXA) and inflammatory markers were measured using multiplex assay. The majority of survivors had been diagnosed with ALL or Acute myelogenous leukemia (AML).

HCT survivors had higher IL-6 levels in ① [mean 5.7 pg/ml (95% CI 4.0-8.2)], ② [mean 4.5 pg/ml (95%CI 3.5-5.9)], and ③ [adjusted mean 5.2 pg/ml (95% CI 3.4-7.9)] compared to their siblings [adjusted mean 3.4 pg/ml (95% CI 2.7-4.3)]. TNF-α levels were slightly but not significantly increased in the three groups [adjusted mean 1.8 pg/ml (95% CI 1.7-2.0) in all groups] compared to the siblings [mean 1.7 pg/ml (95% CI 1.5-1.8)].
Although no differences were found in BMI between survivors and siblings, HCT survivors showed significantly higher PFM in ① [adjusted mean 35.2 (92% CI 32.1-38.4)], ② [adjusted mean 34.4 (95% CI 31.4-37.4)], and ③ [adjusted mean 34.9 (95% CI 31.4-38.4)] compared to siblings [adjusted mean 29.6 (95% CI 26.7-32.4)]. Total LBM was decreased in ① [adjusted mean 34.4 kg (95% CI 31.0-37.7)] and ② [adjusted mean 36.9 kg (95% CI 34.1-37.9)] compared to their siblings [adjusted mean 47.8 kg (95% CI 41.2-50.0)]. As described, the means in body composition and inflammatory markers were reported and compared between HCT survivors and siblings in this study, but no analyses were performed to investigate the association between body composition (PFM and LBM) and inflammatory markers (IL-6 and TNF-α).

Table 2. *Outcomes of included studies*

First author Year	Type of lifestyle behavior	Type of inflammatory marker	Outcome definition	Statistical Analysis	Results	Risk of Bias
Blair C, 2014	pGJ vs cAJ	MPO Hs-CRP	Adjusted differences in MPO and hs-CRP levels after pGJ supplementation compared to cAJ supplementation	Mixed ANCOVA Fixed effects: period, group, juice, baseline Random effects: Subjects within group	Adjusted differences (95% CI) pGJ vs cAJ: <u>MPO</u> 0.92 ng/ml (0.82-1.03), p=0.15 <u>Hs-CRP</u> 1.34 mg/l (0.69-2.62), p=0.37	Selection bias: Unclear risk Attrition bias: Low risk Detection bias: Unclear risk Confounding: Low risk
Ketterl TG, 2018	Body composition (BMI, PFM, LBM)	IL-6 TNF-α	Difference in BMI, PFM, LBM, IL-6 and TNF-α levels in HCT survivors in different treatment groups compared to matched siblings. HCT treatment groups: ① TBI + CNS radiation (n=31) ② TBI (n=85) ③ Chemotherapy (n=35)	Multivariate linear regression Covariates: age, sex, ethnicity, Tanner score Separate analyses for GVHD	Adjusted means (95% CI) HCT survivors per treatment group vs siblings: <u>IL-6</u> ① 5.7 pg/ml (4.0-8.2), **p=0.005** ② 4.5 pg/ml (3.5-5.9), **p=0.002** ③ 5.2 pg/ml (3.4-7.9), **p=0.019** Siblings: 3.4 pg/ml (2.7-4.3) <u>TNF-α</u> ① 1.8 pg/ml (1.7-2.0), p=0.061 ② 1.8 pg/ml (1.7-2.0), p=0.056 ③ 1.8 pg/ml (1.7-2.0), p=0.303 Siblings: 1.7 pg/ml (1.5-1.8) <u>LBM</u> ① 34.4 kg (31.0-37.7), **p<0.001** ② 36.9 kg (34.1-39.7), **p<0.001** ③ 45.6 (41.2-50.0), p=0.262 Siblings: 47.8 kg (45.0-50.5) <u>PFM</u> ① 35.2 (32.1-38.4), **p<0.001** ② 34.4 (31.4-37.4), **p<0.001** ③ 34.9 (31.4-38.4), **p=0.005** Siblings: 29.6 (26.7-32.4)	Selection bias: Low risk Attrition bias: Low risk Performance bias: Unclear risk Detection bias: Unclear risk

**Both cases and controls were cancer survivors, randomly assigned to the intervention or control group. ** Cases were 151 HCT survivors and 92 of their siblings were defined as controls. HCT survivors were categorized in three groups based on previous cancer treatment, being ① TBI with CNS radiation, ② TBI without CNS radiation and ③ chemotherapy. Abbreviations: BMI= Body Mass Index; GVHD= Graft-versus-host disease; HCT= Hematopoietic Cell Transplantation; HS= High sensitivity C-reactive protein; IL=Interleukin; LBM=Lean Body Mass; MPO= Myeloperoxidase; NR=Not Reported; PFM=Percent Fat Mass; RCT= Randomized Controlled Trial; SD=Standard Deviation; TBI= Total Body Irradiation; TNF=Tumor Necrosis Factor. p-values<0.05 are shown in bold.*

Risk of bias

Selection bias

Selection bias was assessed by evaluating the randomization procedure and the selection procedure. In the RCT of Blair and colleagues (28), equal allocation at randomization was reported for the juice group sequence, balanced in regard to baseline characteristics. It was observed that the group starting with cAJ had higher numbers of hematopoietic malignancy survivors and a higher BMI. No information was available on concealment of the allocation sequence. Based on the provided information and randomization procedure, the risk of selection bias of the RCT was estimated to be low.

For the study of Ketterl and colleagues (29), the risk of selection bias was unclear. To illustrate, participants were recruited using transplantation databases of two hospitals, which could have led to non-random sampling. Additionally, although the authors reported exclusion of three participants after consent, because of undiagnosed Type 2 diabetes, high hypertension, or unspecified medical issues, it is unclear if other candidates with these conditions were included or excluded.

Attrition bias

Risk of attrition bias was assessed by looking at the number of participants in which the outcome was measured and loss to follow-up. The risk of attrition bias was considered low in the study of Blair et al (28), as inflammatory marker measurements were performed in all subjects that were randomized into the juice groups (6 dropouts in run-in period). Risk of attrition bias was also considered low in the study of Ketterl et al (29), as both inflammatory markers and body composition was measured in the entire study population.

Detection- and Performance bias

The risk of detection- and performance bias was assessed by evaluating blinding procedures of outcome assessors and participants/personnel, respectively. Both studies had an unclear risk of detection bias, as there was no report of blinding in either study. There was also no mention of blinding of participants or personnel in the RCT of Blair et al (28).

Risk of Confounding

The risk of confounding was assessed by evaluating if important possible confounders were considered, such as cancer type, treatment, age, and history of inflammatory diseases. The risk of confounding was estimated as low in the study of Ketterl (29), since almost all described variables (Supplemental table 9.2) were taken into account, including adjustments for attained age (but not age at diagnosis), sex, ethnicity, Tanner score and graft-versus-host disease, along with separate analyses in three different cancer treatment groups.

7 Discussion

This systematic review gave an overview of the current knowledge on lifestyle and inflammation in CAYA cancer survivors. Of two studies who fulfilled the inclusion criteria, one reported on the effect of pGJ vs cAJ on MPO and hs-CRP in survivors of various childhood cancers (28), whereas the other investigated differences of HCT survivors and siblings in terms of body composition and inflammatory markers (IL-6 and TNF-α) (29). The first study showed no differences in inflammatory markers after pGJ supplementation as compared to cAJ in survivors of childhood cancer. The second study showed that they had higher IL-6 levels and higher PFM compared to their siblings, whereas BMI was similar.

The intervention with pGJ was performed based on the previously established beneficial effects of flavonoids, components in red wine and pGJ, on vascular endothelial function and inflammation. To illustrate, flavonoid intake has been associated with decreased hs-CRP and IL-18 levels in healthy adults, which likely is mediated by anti-oxidant properties of flavonoids (30–33). As childhood cancer survivors are at increased risk of developing cardiovascular diseases and chronic inflammation, it is plausible to assume that pGJ might decrease inflammatory markers in childhood survivors. There are several possible explanations for the fact that Blair and colleagues (28) did not find a significant effect of pGJ supplementation on inflammatory markers. First, the small sample size of the RCT (n=24) is likely too small to detect significant differences. Second, the treatment period (4 weeks) might have been too short to adequately assess the effect of pGJ compared to cAJ. Third, the levels of inflammatory markers of the childhood cancer survivors were not compared to healthy controls, which makes it unclear if there was any room for improvement of these markers and what the sole effect of pGJ was. Fourth, contamination within supplementation periods (i.e. pGJ consumption during cAJ period) could have occurred and skewed the results. Therefore, although this cross-over RCT was well-designed, it would be relevant to evaluate the effects of pGJ supplementation in a larger sample, for a longer treatment period, and with healthy controls.

The observational study of Ketterl and colleagues was designed to further elucidate the pathophysiology underlying the increased risk of cardiovascular- and metabolic conditions in HCT survivors. Past research showed, that although childhood cancer survivors and HCT survivors are often not obese, they have higher total fat mass, reduced lean fat mass and lower insulin sensitivity, which are risk factors for diabetes and cardiovascular diseases (34). It was hypothesized that visceral adipose tissue-produced inflammatory factors might play an important role in the increased prevalence of cardiometabolic diseases in HCT survivors and other groups of childhood cancer survivors (35). The study of Ketterl and colleagues provided evidence that this hypothesis has merit, as fat mass and inflammatory markers were both increased in HCT survivors compared to their siblings. However, a major limitation of this study was the lack of statistical analyses confirming an association between increased fat mass and increased inflammatory markers, which limits the usefulness of these results in light of the aim of this review. Furthermore, due to the cross-sectional design, no conclusions can be drawn about the sequence of events in the post-HCT period.

Thus, the presented studies did not demonstrate an association between lifestyle behaviors and inflammatory markers in childhood cancer survivors, but the study of Ketterl (29) pointed towards increased total lean body mass or decreased PFM as a possible modulator of chronic inflammation, which in turn is one of the causes of the cardiometabolic risk profile of childhood cancer survivors. It should be noted that low lean body mass does not need to be related to lifestyle and can be caused by treatment-related endocrine disturbances such as growth-hormone deficiency (36). Nonetheless, physical activity has been previously associated with increased lean body mass in childhood cancer

survivors, suggesting that exercise might be a viable target to delay late effects by increasing lean body mass and consequently limiting chronic inflammation (37). In adult cancer survivors, we already have considerable evidence of a positive effect, not just of conventional exercise, but also yoga and tai-chi, on low-grade inflammation (38–43). More broadly, various lifestyle behaviors, ranging from dietary compounds to mindfulness training, have been associated with less chronic inflammation in adult cancer survivors (44–46). It is important to stress, that although randomized trials have shown promising effects of a healthy lifestyle on inflammation and quality of life, it is often difficult to make and sustain lifestyle changes. The observation that cancer survivors generally poorly adhere to lifestyle guidelines highlights the need of new implementation strategies and active support for survivors in order to achieve durable lifestyle changes (19,47–49)**.**

As demonstrated, the potential of lifestyle behavior has gained attention in adult cancer survivors, but only very few observational studies and trials have been performed to examine the influence of lifestyle on inflammation in childhood cancer survivors. This review discussed the studies describing lifestyle behaviors and inflammation in childhood cancer survivors, and as these two studies were different in many aspects, such as the design, type of lifestyle behavior and type of inflammatory marker, it is not possible to apply these results to the general population of childhood cancer survivors. Furthermore, the quality of the studies was difficult to assess, as insufficient information was provided on sequence allocation, characteristics of excluded participants and blinding procedures. Strengths of both studies, however, were the complete follow-up data and adjustment of relevant variables in the analyses. Taken together, although both studies provided insight into the role of lifestyle on inflammation, the results should be interpreted with caution.

In conclusion, only two studies reporting lifestyle behaviors in relation to inflammation in childhood cancer survivors were identified and described in this review, indicating that we are only at the very beginning of uncovering the role of lifestyle in reducing low-grade systemic inflammation and hopefully delaying the development of chronic diseases in childhood cancer survivors. Therefore, new observational studies and trials covering multiple aspects of lifestyle are necessary to unravel its anti-inflammatory effect in preventing treatment-related late effects in childhood cancer survivors.

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9 Supplemental data

9.1 Search strategy PubMed

1. Cancer (all age groups):

(((((("nervous system neoplasm*" OR leukemias OR leukemia OR leukemi* OR leukaemi* OR aml OR anll OR lymphoma OR lymphom* OR hodgkin* OR T-cell OR B-cell OR non-hodgkin OR sarcoma OR sarcom* OR Ewing* OR osteosarcom* OR wilms* OR nephroblastom* OR neuroblastom* OR rhabdomyosarcom* OR teratom* OR hepatom* OR hepatoblastom* OR PNET OR medulloblastom* OR PNET* OR "neuroectodermal tumors primitive" OR retinoblastoma OR retinoblastom* OR meningiom* OR gliom* OR neoplasms OR "brain tumor*" OR "brain neoplasm*" OR "central nervous system neoplasm*" OR "central nervous system tumo*" OR "central nervous system cancer*" OR "brain cancer*" OR "brain neoplasm*" OR "intracranial neoplasm*" OR "leukemia lymphocytic acute*") OR (Cancer OR cancers OR cancer* OR oncology OR oncolog* OR neoplasm OR neoplasms OR neoplasm* OR carcinoma OR carcinom* OR tumor OR tumour OR tumor* OR tumour* OR tumors OR tumours OR malignan* OR malignant OR hematooncological OR hemato oncological OR hemato-oncological OR hematologic neoplasms OR hematolo*))

AND (("Pediatric oncolog*" OR "Paediatric oncolog*" OR "Childhood cance*" OR "Childhood tumo*" OR "Childhood neoplasm*") OR (Infan* OR toddler* OR minors OR minors* OR boy OR boys OR boyfriend OR boyhood OR girl* OR kid OR kids OR child OR child* OR children* OR schoolchild* OR schoolchild OR school child[tiab] OR school child*[tiab] OR adolescen* OR juvenil* OR youth* OR teen* OR under*age* OR pubescen* OR pediatrics[mh] OR pediatric* OR paediatric* OR peadiatric* OR school[tiab] OR school*[tiab] OR young adult[mh] OR young adult OR adult OR adults OR adult* OR adulthood OR "Adult"[Mesh])))

2. Late effects / survival

AND (("late effect*" OR "long term" OR "later side effect*" OR "symbolic interactionism" OR surviv* OR survival* OR aftercare OR "continuum of care" OR "maintenance therapy" OR "outpatient treatment" OR "partial hospitalization" OR "posttreatment followup") OR (Survivor OR survivors OR survivor* OR long term survivor OR long term survivors OR long term survivor* OR survivo* OR long term survival[tiab] OR survival[mh])))

3. Life style (including exercise, diet, smoking, alcohol intake, vitamin D and stress)

AND ("Life Style"[Mesh] OR lifestyle OR "health behavior" OR lifestyle* OR "life style" OR "health promotion" OR "client education" OR "health education" OR "weight control" OR "weight gain" OR "weight loss" OR "weight control*" OR "body weight maintena*" OR "weight loss" OR overweight OR obesit* OR "Exercise"[Mesh] OR "Exercise Therapy"[Mesh] OR "Diet, Reducing"[Mesh] OR exercise OR "physical activity" OR sports OR swimming OR "physical exercise*" OR exercis* OR walking OR training OR "sedentary behavior" OR "tobacco smoking" OR "smoking cessation" OR "smoking cessat*" OR smoking OR smoke OR "alcohol drinking patterns" OR "drinking behavior" OR alcoholism OR alcohol OR "alcohol drinking" OR "alcohol intake" OR "food intake" OR nutrition OR diet* OR "dietary chang*" OR diet OR eating OR "dietary intake" OR fruit OR vegetable OR nutrition OR "Vitamin D"[Mesh] OR Stress* OR "Stress relaxation"))

4. Inflammation

AND ((Inflammation OR "Innate Inflammatory Response*" OR "Inflammatory Response, Innate" OR "Chronic inflammation*" OR "Low-Grade Inflammation" OR "Inflammation Marker*" OR "Inflammatory Marker*" OR Inflamm-Aging OR Inflamm-aging OR Inflammageing) OR ("C-reactive protein" OR "C-reactive protein levels" OR "C-Reactive Protein"[Mesh] OR "Interleukins"[Mesh] OR "Interleukin") OR ("Tumor-necrosis factor" OR "Acute-Phase Proteins"[Mesh]))

The searches were combined in PubMed as 1 AND 2 AND 3 AND 4. The search was performed on 3 June 2022.

9.2 Risk of bias criteria for observational studies

	Internal validity
Study group	**Selection Bias (is the study group representative?)** Low risk if: The study group consisted of more than 90% or the original cohort of eligible patients for the review *or* It was a random sample with respect to cancer type, cancer treatment, age at primary diagnosis, attained age and history of inflammatory disease
Follow-up	**Attrition bias (is the follow-up adequate?):** Low risk if: The outcome was assessed for more than 90% of the study group
Outcome	**Detection bias (are the outcome assessors blinded for important determinants related to the outcome?)** Low risk if: The outcome assessors were blinded for important determinants related to the outcome
Risk estimation	**Confounding (are the analyses adjusted for important confounders?)** Low risk if: Important prognostic factors (i.e. cancer type, cancer treatment, age at primary diagnosis, attained age and history of inflammatory disease) were taken adequately into account

9.3 Risk of bias criteria for randomized controlled trials

	Internal validity
Study group	**Selection Bias (is the study group representative?)** Low risk if: There was random sequence allocation and allocation concealment
Follow-up	**Attrition bias (is the follow-up adequate?):** Low risk if: The outcome was assessed for more than 90% in each treatment arm
Outcome	**Performance bias (are the participants and personnel blinded from knowledge of which intervention was received?)** Low risk if: The participants and personnel were blinded from knowledge of which intervention was received **Detection bias (are the outcome assessors blinded from knowledge of which intervention was received?)** Low risk if: The outcome assessors were blinded from knowledge of which intervention was received

9.4 Association between lifestyle behaviors and inflammation

Blair C et al. Feasibility and Preliminary Efficacy of the Effects of Flavanoid-Rich Purple Grape Juice on the Vascular Health of Childhood Cancer Survivors: A Randomized, Controlled Crossover Trial. Pediatr Blood Cancer 2014;61(12):2290-2296

Study design & Main study objective	**Participants and relevant characteristics**	**Relevant results**	**Additional remarks & Risk of Bias**
1. Study design Randomised controlled crossover trial: intervention versus control substance with wash-out periods 2. Study objective The primary aim of this trial was to evaluate the effects pGJ supplementation on microvascular endothelial function and biomarkers of systemic and vascular oxidative stress and inflammation. 3. Additional study characteristics, if relevant - Study design: participants started with a 4-week run-in period where they refrained from consumption of any juice (drinks), wine or grapes. After randomization, participants drank 6 ounces of pGJ (intervention) or cAJ (control) daily. After a 4-week wash-out period, participants consumed 6 ounces of the other supplementary drink. Outcome measures were performed prior and after each supplementation period. - Methodology analysis: Changes in markers from baseline between pGJ and cAJ were analysed with mixed ANCOVA (period, group, juice, baseline as fixed effects, subjects within group as random effect). MPO and hs-CRP were log-transformed prior to analyses. - follow-up: not reported	1. Type and number of participants Childhood cancer survivors, aged 10-22 years old, diagnosed with any cancer, off treatment more than 3 years, which recently completed an NIH funded study on metabolic syndrome or received healthcare at the Long-Term Follow-up Clinic. Exclusion criteria were pregnancy, start of oral contraceptives <3 months before study, smoker, antibiotic use <2 weeks before study, diabetes. 119 candidates were contacted, 30 completed the run-in period and 24 completed the study. 2. Age (at diagnosis) of participants Current mean age (years): 16.4 (range 13.7-17.2) Age at cancer diagnosis(years): 3.6 (1.5-6.1) Time since cancer treatment (years): 8.5 (6.4-13.0) 3. Number of participants per diagnosis Blood cancer n=12 (50%) ALL n=9 AML n=1 Hodgkin lymphoma n=1 Solid tumor n=12 (50%) CNS tumor n=3 Bone tumor n=2 Retinoblastoma n=2 Germ cell tumor n=2 Neuroblastoma n=1 Hepatoblastoma n=1 Soft tissue sarcoma n=1 5. Additional participants characteristics, if relevant Sex: Male n=17 (71%) Female n=7 (29 %) Country: USA	Type of lifestyle behavior This randomized controlled crossover trial was a lifestyle dietary intervention, investigating the effect of supplementation with pGJ on vascular health and inflammatory markers in, as opposed to cAJ, in periods of 4 weeks. Outcome definition Inflammatory biomarker assessment was performed using plasma samples, where myeloperoxidase (MPO) and high sensitivity C-reactive protein (hs-CRP) were measured with an enzyme-linked immunosorbent assay. Results: Unadjusted log-transformed median values (IQR) of MPO and Hs-CRP before and after pGJ and cAJ supplementation were separately reported without p-values. Unadjusted Median (IQR) values in pGJ Pre-intervention MPO: 117.3 ng/ml (98.6,138) Post-intervention MPO: 107.0 ng/ml (91.7,131) Pre-intervention Hs-CRP: 0.19 mg/l (0.09,0.41) Post-intervention Hs-CRP 0.33 mg/l (0.15,0.73) Unadjusted Median (IQR) values in cAJ Pre-intervention MPO: 116.2 ng/ml (92.6,142) Post intervention MPO: 116.1 ng/ml (99.1,144) Pre-intervention Hs-CRP: 0.24 mg/l (0.07,0,55) Post-intervention Hs-CRP: 0.24 mg/l (0.11,0.85) The difference in effect of pGJ compared to cAJ on MPO and HS-CRP were reported as adjusted differences (95% CI) from the mixed-effects ANCOVA model. P values represent comparison between pGJ supplementation and cAJ supplementation. No significant changes were observed in inflammatory markers from baseline, compared between pGJ and cAJ groups. Adjusted differences (95% CI) of	Study strengths - Efficient cross-over design - Run-in period in which both groups refrained from drinking any juices - Compliance assessed - Mixed ANCOVA statistical analyses - First intervention of pGJ in childhood cancer survivors Limitations - Relatively short time of trial - Possible contamination of groups (not sure if pGJ only drank grape and never apple juice) - No follow-up of the effect on long-term Risk of Bias Selection bias: Low risk Subjects were randomized to the juice sequence (pGJ vs cAJ first) in equal allocation, balanced in regard to baseline characteristics. More hematopoietic malignancy survivors and higher BMI's randomized to start with cAJ. Attrition bias Low risk 6 dropouts in run-in period, but none in the intervention periods. Inflammatory

		inflammatory biomarkers after pGJ vs cAJ supplementation MPO: 0.92 ng/ml (0.82-1.03), p=0.15 Hs-CRP: 1.34 mg/l (0.69-2.62), p=0.37	markers were assessed in all 24 subjects (some weighted estimate due to unsuccessful blood draw in 1/4 visits). Performance bias Unclear risk No report of blinding of participants or personnel Detection bias Unclear risk No report of blinding of outcome assessors

Ketterl TG et al. Adipokines, Inflammation, and Adiposity in Hematopoietic Cell Transplantation Survivors. Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant. 2018 Mar;24(3):622–6

Study design & Main study objective	**Participants and relevant characteristics**	**Relevant results**	**Additional remarks & Risk of Bias**
1. Study design Observational study with cross-sectional design in 4 groups (3 HCT survivor groups and their siblings) 2. Study objective The primary aim of this trial was to evaluate inflammatory markers, adipokine levels, body composition and adiposity in childhood cancer survivors who underwent hematopoietic cell transplantation (HCT) compared to their siblings. 3. Additional study characteristics, if relevant - Methodology analysis: HCT survivors were categorized in 3 treatment groups (TBI with or without CNS irradiation and chemotherapy without irradiation). Changes in markers, compared between the survivor treatment groups and siblings, were analyzed using multiple linear regression, with age, sex, ethnicity, and Tanner score as covariates. - follow-up: not reported	1. Type and number of participants Childhood cancer survivors of a primary hematologic malignancy, 21 years of younger, underwent HCT 2 or more years ago, currently in remission, at least 9 years at the time of examination. Siblings were at least 9 years at examination, never had cancer and matched to HCT survivor by age and sex. 339 survivors were contacted, 154 survivors and 92 siblings provided consent. 151 survivors enrolled and completed the study. 2. Age (at diagnosis) of participants Current mean age (SD) (years) Survivors: 24 (6) Siblings: 24 (0.7) Age at cancer diagnosis: not reported 3. Number of participants per diagnosis ALL n=47 (31.1%) AML n=54 (35.7%) CML n=15 (9.9%) Hodgkin lymphoma n=12 (8%) Myelodysplastic syndrome n=13 (8.6%) Other hematological malignancy	Type of lifestyle behavior This observational study looked at body composition (BMI, percent fat mass (PFM) and Total Lean Body Mass (LBM)) in relation to inflammatory markers and adipokine levels. These were compared in HCT survivors in 3 different treatment groups and their siblings. Outcome definition Fat mass and lean body mass measurements were measured using DXA. Biomarker assessment was performed using plasma samples, where the IL-6 and TNF-α were measured using the Luminex multiplex assay. Results: Adjusted Means (95% CI) for BMI, total LBM, PFM, IL-6 and TNF-α in survivors treated with TBI, with and without CNS radiation, and chemotherapy. P values represent comparison with the control group (siblings). PFM and IL-6 were significantly increased in survivors (in all treatment groups) compared to their siblings, whereas BMI was not significantly different. LBM was lower in the two TBI treatment groups compared to siblings. Survivors TBI + CNS Radiation vs Siblings BMI: 23.3 kg/m^2 (20.9-20.5), p=0.162 *(Sibling: 24.6 kg/m^2 (22.6-26.7))* Total LBM: 34.4 kg (31.0-37.7), **p<0.001**	Study strengths - Large sample size - Reliable and valid assessment of the inflammatory markers - Age & sex-matched siblings as controls - Analyses with different categories of treatment Limitations - Low response rate, influences representativity - DXA is not most sensitive method for evaluating adiposity - Group sizes for treatment groups not equal - No conclusions possible about different body composition as an underlying factor in higher IL-6 levels in survivors Risk of Bias Selection bias: Unclear risk

	n=10 (6.6%) <u>5. Additional participants characteristics, if relevant</u> Sex Survivors Male n= 87 (57.6%) Female n=64 (42.4 %) Siblings Male n=49 (53.6%) Female n=43 (46.4 %) Country: USA Cancer treatment groups TBI + CNS radiation n=31 TBI – CNS radiation n=85 Chemotherapy n=35	*(Sibling: 47.8 kg (45.0-50.5))* PFM: 35.2 (32.1-38.4), **p<0.001** *(Sibling: 29.6 (26.7-32.4))* IL-6: 5.7 pg/ml (4.0-8.2), **p=0.005** *(Sibling: 3.4 pg/ml (2.7-4.3))* TNF-α: 1.8 pg/ml (1.7-2.0), p=0.061 *(Sibling: 1.7 pg/ml (1.5-1.8))* Survivors TBI – CNS Radiation vs Siblings BMI:23.4 kg/m^2 (121.4-25.4), p=0.05 *(Sibling: 24.6 kg/m^2 (22.6-26.7))* Total LBM: 36.9 kg (34.1-39.7), **p<0.001** *(Sibling: 47.8 kg (45.0-50.5))* PFM: 34.4 (31.4-37.4), **p<0.001** *(Sibling: 29.6 (26.7-32.4))* IL-6: 4.5 pg/ml (3.5-5.9), **p=0.002** *(Sibling: 3.4 pg/ml (2.7-4.3))* TNF-α: 1.8 pg/ml (1.7-2.0), p=0.056 *(Sibling: 1.7 pg/ml (1.5-1.8))* Survivors Chemotherapy vs Siblings BMI:25.8 kg/m^2 (23.3-28.3), p=0.321 *(Sibling: 24.6 kg/m^2 (22.6-26.7))* Total LBM kg: 45.6 (41.2-50.0), p=0.262 *(Sibling: 47.8 kg (45.0-50.5))* PFM: 34.9 (31.4-38.4), **p=0.005** *(Sibling: 29.6 (26.7-32.4))* IL-6: 5.2 pg/ml (3.4-7.9), **p=0.019** *(Sibling: 3.4 pg/ml (2.7-4.3))* TNF-α: 1.8 pg/ml (1.7-2.0), p=0.303 *(Sibling: 1.7 pg/ml (1.5-1.8))*	HCT Survivors were selected from transplantation databases (2 hospitals). Not clear if candidates who did not consent had different characteristics than the included ones. 3 participants excluded based on medical condition (1x T2M, 1x hypertension, 1x unspecified issues), but no further details given. Attrition bias: Low risk Body composition outcomes and inflammatory markers assessed in the entire study population Detection bias: Unclear risk No report of blinding of outcome assessors Confounding: Low risk Adjusted for attained age, sex, ethnicity, and Tanner score. Separate analyses to adjust for GVHD (no associations found). Therefore, we estimated the risk of confounding as low due to the adjustment of almost all established variables and categorization into treatment groups.

Abbreviations: ALL= Acute Lymphoblastic Leukemia; AML= Acute Myelogenous Leukemia; BMI= Body Mass Index; cAJ= Clear Apple Juice; CI= Confidence Intervals; CML= Chronic Myelogenous Leukemia; CNS= Central Nervous System; DXA= Dual-Energy X-Ray Absorptiometry; GVHD= Graft-versus-host disease; HCT= Hematopoietic Cell Transplantation; HS-CRP=High Sensitivity C-reactive protein; IL=Interleukin; IQR= Interquartile range; LBM=Lean Body Mass; MPO= Myeloperoxidase; NR=Not Reported; PFM=Percent Fat Mass; pGJ= Purple Grape Juice; RCT= Randomized Controlled Trial; SD=Standard Deviation; TBI= Total Body Irradiation; TNF=Tumor Necrosis Factor. p-values<0.05 are shown in bold.