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Changes in Plasma Bioactive Lipids and Inflammatory Markers during a Half-Marathon in Trained Athletes

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Abstract: Background: Exercise may affect lipid profile which in turn is related to inflammation, although changes of ceramides, diacylglycerols-DAG and sphingomyelin-SM and their relationship with inflammatory parameters following a half-marathon have never been examined. Methods: Ceramides, DAG and SM, and markers of inflammation (soluble fractalkine-CX3CL1, vascular endothelial growth factor-VEGF, interleukin6-IL-6 and tumor necrosis factorα-TNFα) were evaluated in trained half-marathoners before, post-race (withdrawal within 20 min after the race end) and 24 h after. Results: IL-6 and CX3CL1 increased immediately after the race, returning to baseline after 24 h. Total ceramides and total DAG significantly decreased post-race. Several ceramide classes decreased after exercise, while only one of the DAG (36:3) changed significantly. Total SM and specific species did not significantly change. Conclusion: Some inflammatory parameters (IL-6 and CX3CL1) transiently increased after the race, and, being reversible, these changes might represent a physiological response to acute exercise rather than a damage-related response. The decrease of specific lipid classes, i.e., DAGs and ceramides, and the lack of their relationship with inflammatory parameters, suggest their involvement in beneficial training effects, opening promising research perspectives to identify additional mechanisms of aerobic exercise adaptation.

Keywords: exercise; ceramides; cytokines; diacylglycerol; biomarkers

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1. Introduction

Half-marathon (21.0975 km) is an increasingly popular recreational activity. Its diffusion is in part related to the fact that half-marathon requires less powerful training than the marathon [1]. Nonetheless, one reason for its importance in terms of clinical risk is that half-marathon is performed by a growing number of people in the world, although it still remains a powerful training, which might potentially retain adverse health repercussions. In fact, studies focusing on the role of exercise and inflammatory response have underlined that several cytokines increased after a competitive marathon [2]. In this context, it is interesting to assess the role of sphingolipids, bioactive lipids that regulate diverse cell functions, since the activity of sphingomyelinases (sphingolipid metabolizing enzymes) is increased under inflammation and oxidative stress [3]. Thus, ceramides have been associated with oxidative stress status and inflammatory processes [3]. However, changes of ceramide concentration after a half-marathon race have never been examined. Moreover, to the best of our knowledge, the relationship that may exist between bioactive lipids and markers of inflammation/immune response has never been studied in trained subjects following acute physical exertion such as a half-marathon. In this context, how changes in the balance between inflammatory mediators, immune response or lipid may contribute to the response in the post-race phase and affect the health status of subjects participating in such

events also remains unclear. Thus, we aimed to evaluate levels of bioactive lipids related to inflammation signaling pathways, and to this purpose, we studied plasma ceramides, diacylglycerol and SM. These lipids can modulate the activity of intracellular enzymes (e.g., those involved in insulin signaling). In detail:

- Ceramides exert their influence in cellular stress response, inflammatory processes, apoptosis and signaling pathways. They are also accumulated in skeletal muscle, promoting insulin resistance and oxidative stress, contributing to the onset and development of cardiometabolic diseases and renal dysfunction [4]. Several inflammatory cytokines may generate ROS and also induce ceramide formation in several cell types [5].
- Diacylglycerols (DAG) act as second messengers affecting signal transduction from many immune cell receptors and can be produced and metabolized through multiple mechanisms. Moreover, DAG induces the hydrolysis of SM to ceramides.
- Sphingomyelins (SM) are reservoirs for other sphingolipids, influencing cell signaling through their structural role in lipid rafts or through the effects of their catabolic mediators (e.g., ceramides) [6]. Changes in SM concentration directly impact cell membrane physiology by modifying its transmission signal.

Thus, we aimed to evaluate the changes in plasma levels of these bioactive lipids in healthy runners performing a half-marathon, at the end of the race and after 24 h recovery, and their associations with new recently proposed and common biomarkers of immune activation, which are:

- The soluble fractalkine CX3CL1, a potent chemoattractant of T cells and monocytes, which has a recognized role in both immune cell migration and adhesion and is involved in many inflammatory processes and diseases [7].
- Vascular endothelial growth factor (VEGF) is a multifactorial cytokine that derives from endothelial cells and pericytes in response to hypoxia, and is implicated in angiogenesis and microvascular hyperpermeability events [8]
- Interleukin-6 (IL-6), which is generated by different cell types (e.g., macrophages, endothelial cells and T cells). The contraction of skeletal muscle may induce the release of IL-6 into the interstitium as well as into blood in response to an exercise burst. Moreover, IL-6 may modulate the immunological and metabolic reactions to exercise [9].
- Tumor necrosis factor alpha (TNFα), an inflammatory cytokine produced by macrophages/monocytes during acute inflammation, which affects different ranges of signaling pathways, including those leading to necrosis or apoptosis [10].

2. Materials and Methods

2.1. Characteristics of the Participants

The studied population included 13 healthy Caucasian trained runners belonging to the "Gruppo Podistico Rossini" enrolled during the 2018 edition of the Pisa half-marathon. Inclusion criteria of athletes were regular training and absence of cardiovascular disease or any other systemic disorder. Preliminarily to the race, each participant was submitted to a questionnaire to obtain demographic and clinical data and training history. Body mass index (BMI) was calculated by height and weight, measured in each subject. Body fat composition (fat-free mass (FFM); fat% and fat mass) was evaluated by TANITA. The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Pisa Ethics Committee, Italy (protocol number for study acceptance 2805). Informed consent was obtained from all subjects enrolled in the study.

2.2. Sample Collection, Preparation and Evaluation of Lipids and Inflammatory Markers

Three blood samples were collected from the peripheral vein of the athletes (in fasting conditions): (1) the day before the race (baseline), (2) within 20 min after the end of the half-marathon (post) and (3) 24 h after the run (24 h, recovery period). Blood samples were immediately centrifuged at 2500 g for 10 min and stored at -80 °C until assayed.

Ceramides, SM and DAG were evaluated from 20 µL of plasma (upon being deproteinized using 200 µL of cold methanol) in 13 healthy Caucasian trained runners.

An aliquot of 20 µL of a mix of internal standards SM (d18:1/17:0), ceramide (d18:1/17:0), (Avanti Polar Lipids, Alabaster, AL, USA); DAG (17:0/17:0) (Larodan Solna SE) was added to the sample before deproteinization. The extract was injected in high performance liquid chromatography (Agilent UHPLC 1290, Santa Clara, CA, USA), coupled with a quadrupole time-of-flight mass spectrometry QTOF (QTOF-MS, Agilent 6540, Santa Clara, CA, USA), equipped with electrospray ionization source (ESI). For liquid chromatography analysis, we used ZORBAX Eclipse Plus C18 2.1 × 100 mm 1.8-micron column at 50 °C (Agilent, Santa Clara, CA, USA). The mobile phase A was made by water with 0.1% formic acid and the mobile phase B was made by isopropanol/acetonitrile (1:1, *v*:*v*) with 0.1% formic acid. Injection volume was 1 µL. Lipids were measured in positive electrospray ionization and identified using an internal spectral library. The data were normalized by internal standard representative lipids present in the sample and the analysis of the peaks was performed with Agilent MassHunter software program [11]. DAG species founded in plasma were 32:0, 32:1, 34:1, 34:2, 36:3 and 36:4, while ceramide species evaluated in plasma sample were 18:1/16:0, 18:1/18:0, 18:1/25:0, 18:1/26:0, 18:1/22:0, 18:1/24:1, 18:1/24:0, 18:0/24:0. The metabocard for ceramide species was reported as supplementary material (supplemental data). Fractalkine CX3CL1, VEGF, IL6 and TNFα were analyzed in 25 µL of plasma by a specific assay (MILLIPLEX MAP Millipore corporation, Billerica, MA, USA) using an integrated multi-analyte detection platform (high-throughput technology MagPix system, Luminex xMAP technology) with combined analyst software (MILLIPLEX®) for the biomarkers quantification developing new curve fitting algorithms and optimizing mathematical methods to minimize fitting errors.

2.3. Statistical Analysis

Data were expressed as mean ± SD. **Repeated-measures ANOVA** was used for compared data from the same subjects measured more than once (baseline, post, 24 h). Correlation analysis was performed by Spearman parametric test to assess the relationship between continuous variables. Post Δ and 24 h Δ values from baseline (differences) and % change at different successive time periods with respect to values observed at baseline were calculated for fractalkine (pg/mL), IL-6 (pg/mL), TNFα (pg/mL) and VEGF-α (pg/mL). Owing to skewness, log transformation of fractalkine and TNFα was used for statistical analyses. Log-transformed values were then back-transformed for data presentation.

Data statistical analyses were performed with the Statview statistical package, version 5.0.1 (SAS Institute, Abacus Concept, Berkeley, CA, USA). A p value of <0.05 was considered statistically significant. Heatmapping was performed using MetaboAnalyst R 1.0.3 (XiaLab at McGill University, Montreal, QC, Canada). The data in heatmaps were analyzed by t test, the algorithm used was the average method and the measure of distance was Euclidean.

3. Results

3.1. Demographic and Training Characteristics

The characteristics of the runners are summarized in Table 1. Each athlete was regularly engaged in marathon training for more than 3 years, 3–7 times/week in 1–2 h/session. Athletes performed a running race over the distance of 21.0975 km. Race time ranged between 1.33 and 1.46 h. No correlation of inflammatory parameters or sphingolipids with demographic and training characteristics was observed. No gender-related differences were observed for all the biomarkers evaluated.

Table 1. Anthropometric measurement of runners and physical activity.

Anthropometric Measurement	Runners N (13)
Age (years)	47 ± 6
Gender (M/F)	7/6
Height (cm)	171.6 ± 2
Weight (kg)	67.5 ± 2.2
BMI (kg/m^2)	21.6 ± 0.7
WAIST (cm)	78.2 ± 3.1
PAS (mmHg)	128.3 ± 5.2
PAD (mmHg)	72.16 ± 2.3
FFM (kg)	55.6 ± 3.6
FAT% (kg)	11.54 ± 1.5
Physical activity	
Day/week of training	4 ± 0.3
Km/week	50.1 ± 4.7
Years of training	6 ± 1
Half-marathon race finish time (min)	105.2 ± 3.7

Abbreviations: BMI: body mass index, PAS: systolic arterial pressure, PAD: diastolic arterial pressure, FFM: free fat mass.

3.2. Lipids Levels and Race-Related Trends

3.2.1. Total Ceramides, DAG and SM

Total ceramides, total DAG and SM at baseline, post and 24 h after the race are reported in Figure 1 (panel A, B and C, respectively). When plasma levels of total ceramides were considered, a significant decrease was observed after the race and after 24 h compared to baseline (12.25 ± 3.0, 8.68 ± 0.62, p = 0.011 vs. baseline; 9.70 ± 0.51, µmol/L, p = 0.059 vs. baseline; baseline, post-race, 24 h, respectively), Figure 1A. DAG total analysis evidenced a significant decrease post-race compared to baseline (53.26 ± 21.83 vs. 36.07 ± 18.75 µmol/L, post-race vs. baseline, p = 0.04; and 51.56 ± 34.25 24 h µmol/L, not significant), Figure 1B. Instead, SM tended to decrease after the race and at 24 h, although not significantly (145.66 ± 44.83; 100.2 ± 8.9; 105 ± 7.31 µmol/L), Figure 1C.

3.2.2. Ceramides, DAG and SM Species

The specific DAG and SM ceramide species at baseline, post and 24 h after the race are shown by heatmaps that provide intuitive visualization of the data table. Each colored cell on the map corresponds to a concentration value in the data file, with samples in rows and compound in columns (supplemental data).

Trends of DAG classes at baseline and after the race are reported in Figure 2. The greatest changes were attributed to the DAG 36:3 that decreased significantly post-race (25.56 ± 10.6 basal vs. 14.89 ± 8.14 post-race p = 0.01; 22.44 ± 8.14 24 h µmol/L). The other DAG species followed the same pattern but did not significantly change: DAG 32:0 (1.06 ± 0.26; 2.32 ± 4.6; 1.21 ± 0.71 µmol/L, baseline, post-race, 24 h, respectively); 32:1 (2.07 ± 0.71; 1.64 ± 0.94; 2.24 ± 1.62 µmol/L); 34:1 (8.22 ± 2.68; 5.85 ± 3.32; 8.66 ± 4.54 µmol/L); 34:2 (12.1 ± 4.85; 8.45 ± 4.76; 12.76 ± 9.39 µmol/L) and 36:4 (4.24 ± 4.13; 2.92 ± 2.04; 4.24 ± 3.95 µmol/L) (Figure 2).

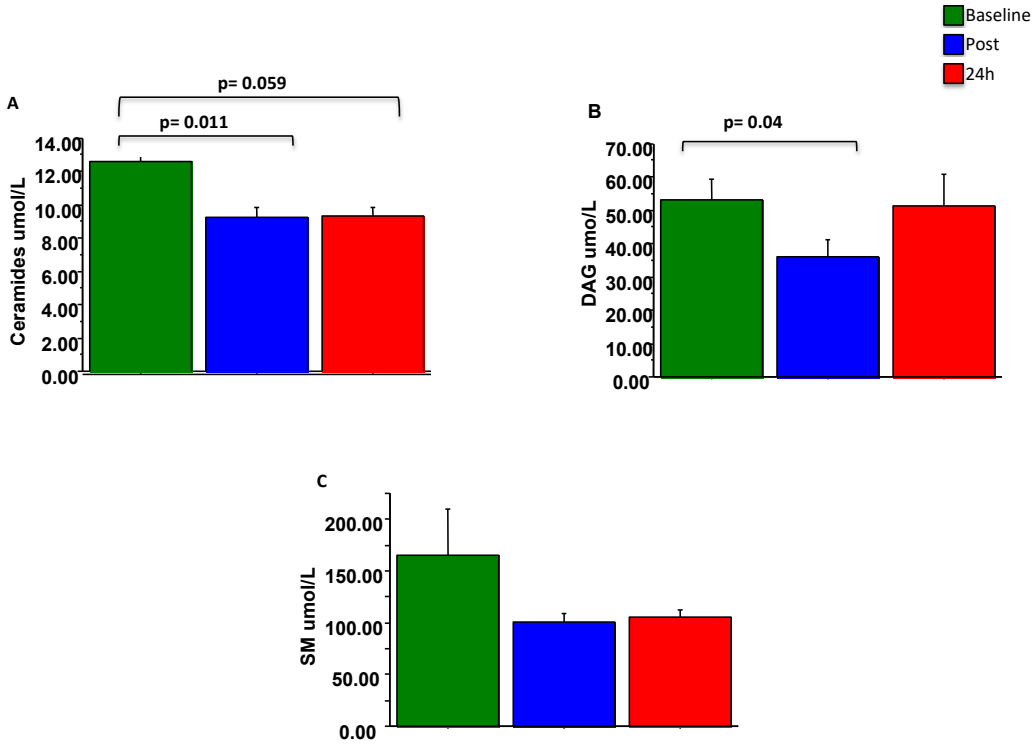

Figure 1. Bar chart reporting mean and SD of total ceramides, total DAG and SM (panel **A**, **B** and **C**, respectively) at baseline, post and 24 h after the race.

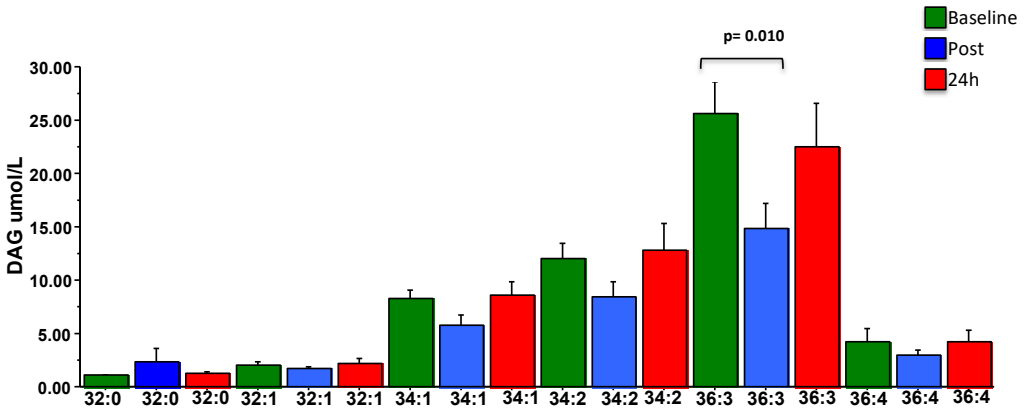

Figure 2. Bar chart reporting the trend of DAG classes as mean and SD at baseline, post and 24 h after the race.

Trends of ceramide species at baseline and after the race are reported in Figure 3. Ceramide species containing long chain fatty acids (18:1/16:0; 18:1/18:0) transiently increased after the race, to significantly decrease at 24 h (0.16 ± 0.06; 0.20 ± 0.05; 0.18 ± 0.04 µmol/L, baseline, post-race, 24 h, respectively); (0.18 ± 0.07; 0.18 ± 0.03; 0.16 ± 0.03 µmol/L) (Figure 3). Instead, ceramides containing very long chain fatty acids, i.e., 18:1/25:0; 18:1/26:0; 18:1/22:0; 18:1/24:1; 18:1/24:0; 18:0/24:0 significantly decreased after the race and at 24 h when compared to baseline (0.82 ± 0.21; 0.55 ± 0.13; 0.62 ± 0.15 µmol/L, baseline, post-race and 24 h, respectively); (0.16 ± 0.05; 0.11 ± 0.03; 0.13 ± 0.03 µmol/L); (1.52 ± 0.35; 1.91 ± 0.50; 1.46 ± 0.43 µmol/L); (3.29 ± 0.91; 2.36 ± 0.60; 2.61 ± 0.38 µmol/L); (5.56 ± 1.4; 1.46 ± 0.43; 4.35 ± 0.95 µmol/L) and (0.17 ± 0.05; 0.12 ± 0.04; 0.13 ± 0.38 µmol/L), Figure 3. SM did not significantly change.

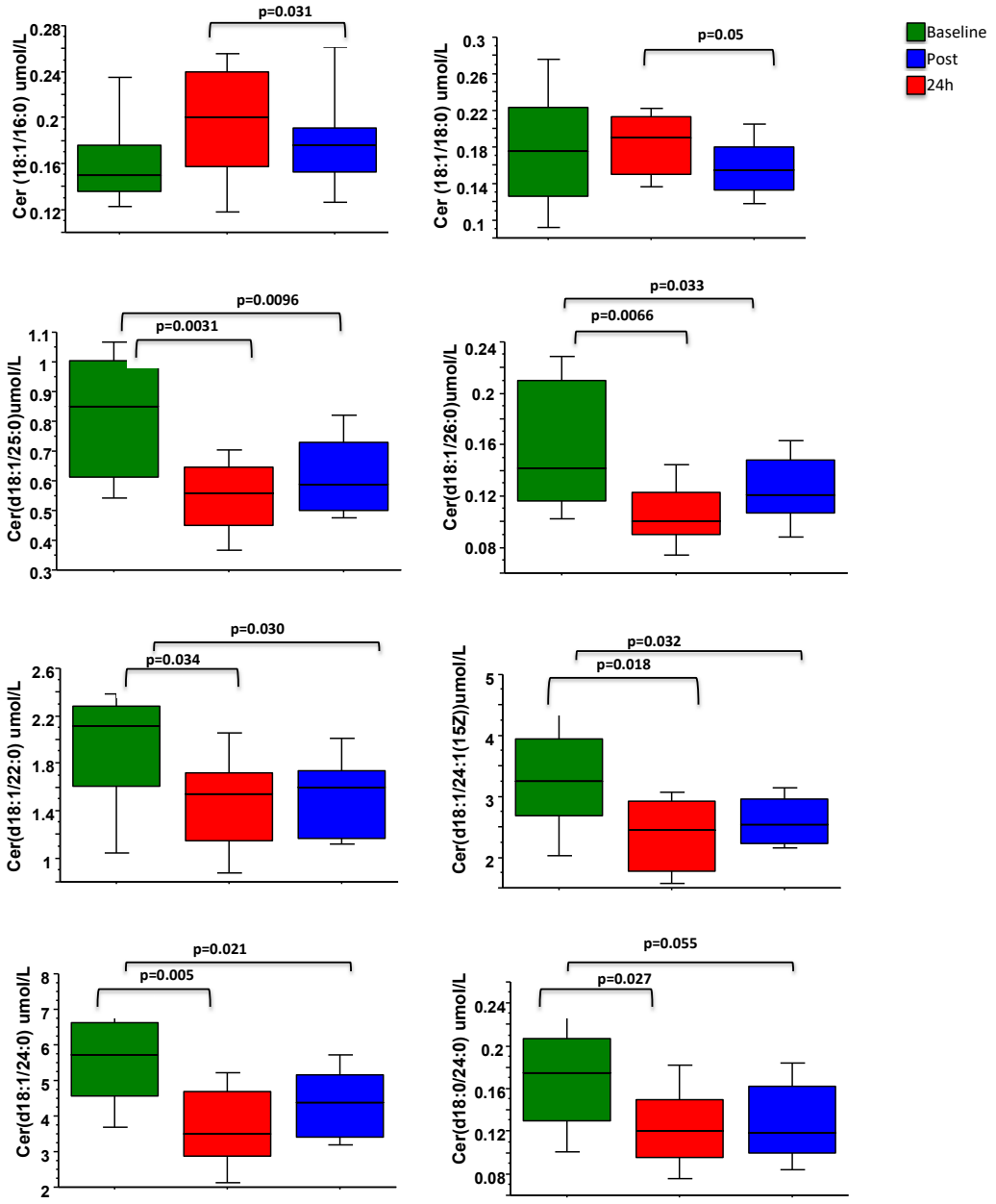

Figure 3. Boxplots with indication of the median, interquartiles and error bars reporting the temporal profile of ceramides are given.

3.3. Inflammatory Levels and Race-Related Trends

Basal plasma levels, post and after 24 h of TNFα, IL-6, fractalkine CX3CL1 and VEGF-A in all runners are reported in Table 2.

Table 2. Plasma values of chemokine and cytokines in runners.

Variables	Mean ± SD		
	Baseline	**Post**	**24 h**
Fractalkine (pg/mL)	143.4 ± 124.9	219.6 ± 126.9	107.7 ± 149.4
IL-6 (pg/mL)	0.7 ± 0.6	9.1 ± 6.9	0.64 ± 0.49
TNFα (pg/mL)	32.4 ± 27.8	35.8 ± 29.1	20.5 ± 24.1
VEGF-A (pg/mL)	186.1 ± 128.4	178.5 ± 161.3	147.6 ± 142.8

Cytokines and chemokines at baseline and after the race are reported in Figure 4. IL-6 and fractalkine CX3CL1 significantly changed soon after the race, decreasing towards baseline values after 24 h (Figure 4A,B). TNFα had the same trend but the increment after the race was not significant (Figure 4C). VEGF-A tended to decrease after the race (Figure 4D), but these changes were not significant when compared to baseline. Table 3 shows the exercise-induced change from baseline, as Δ values (% change) for each variable.

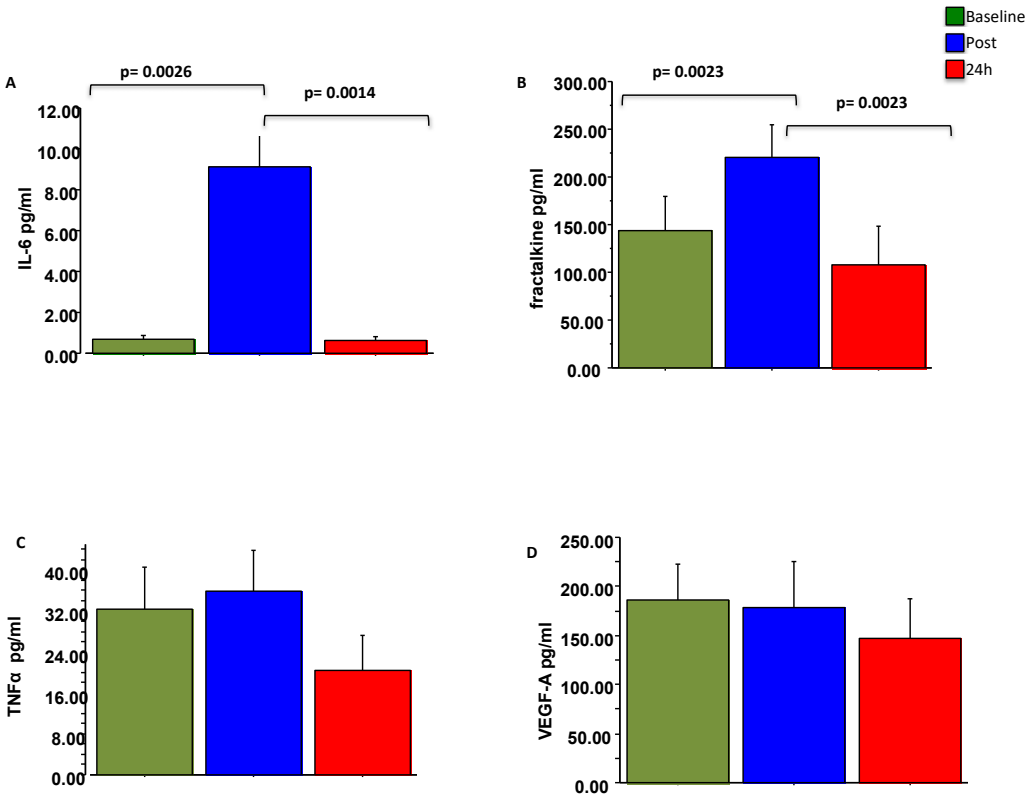

Figure 4. Bar chart reporting mean and SD of cytokines and chemokines at baseline, (**A**) IL-6 (pg/mL), (**B**) Fractalkine (pg/mL), (**C**) TNFα (pg/mL), (**D**) VEGF-A (pg/mL), post and 24 h after the race.

Table 3. Exercise-induced change from baseline, Δ values (% change) in fractalkine, IL-6, TNFα and VEGF-A.

	Pre-Exercise	Post Δ Values from Baseline (% Change)	24 h Δ Values from Baseline (% Change)
Fractalkine (pg/mL)	143.4 ± 124.99	76.2 (53.3%)	−35.7 (−24.9%)
IL-6 (pg/mL)	0.7 ± 0.649	−0.1 (−7.6%)	8.4 (+1208.6%)
TNFα (pg/mL)	32.4 ± 27.844	−11.9 (−36.8%)	3.4 (+10.5%)
VEGF-A (pg/mL)	186.2 ± 128.379	−38.5 (−20.7%)	−7.6 (−4.1%)

Post Δ and 24 h Δ are differences from baseline. % change at different successive time periods with respect to values observed at baseline is reported in brackets.

Interestingly, a strong correlation between basal fractalkine CX3CL1 and TNFα was found at baseline and during recovery (Rho = 0.91 p = 0.0017), Figure 5.

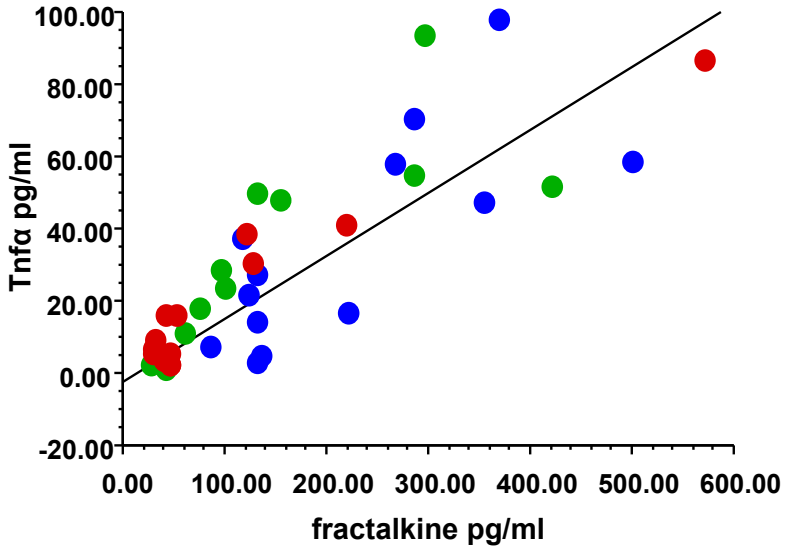

Figure 5. Regression of fractalkine and TNFα in runners (Rho = 0.91 p = 0.0017 24 h). Green dots—baseline, blue dots—post-race, red dots—24 h post-race.

4. Discussion

This is the first study which assessed levels of DAGs and ceramides, and evaluated their relationship with inflammatory parameters in athletes before and after a half-marathon race. Data evidenced the decrease of specific lipid classes, and the lack of relationship between lipids with inflammatory biomarkers, suggesting their possible contribution to exercise-related beneficial effects.

4.1. Demographic and Training Characteristics

The population was similar in demographic and training characteristics; likely, for this reason, we did not observe any correlation concerning inflammatory parameters and sphingolipids. Regarding gender-related differences, it is not clear in literature if ceramides are different in males and females, and neither wheter there is a differential effect of exercise on ceramides in the two sexes. In our population, we did not observe gender-related differences for any of the biomarkers evaluated. Moreover, there is still a lack of a shared consensus on the optimal cutoffs and reference ranges of ceramides to be used. In this context, our data might suggest a lack of need to establish gender-specific reference ranges. However, considering the low number of subjects enrolled in the study, this issue merits further investigation.

Notably, values of inflammatory biomarkers of athletes at baseline did not differ from those observed in a group of 15 sedentary subjects (corresponding to 0.6 ± 3.1 pg/mL for IL-6, 164 ± 145 pg/mL for fractalkine CX3CL1, 23 ± 38 pg/mL for TNFα), except for VEGF-A, which was significantly lower in sedentary subjects (28 ± 61 pg/mL, $p < 0.001$) (unpublished data).

4.2. Lipids Levels and Race-Related Trends

To the best of our knowledge, this is the first report describing half-marathon consequences on circulating sphingolipid metabolism. It is known that exercise may induce modification in plasma levels of numerous circulating biomarkers, inflammatory parameters and lipid metabolites in humans [12] Thus, these changes may affect sphingolipid metabolites as well. In recent years, plasma sphingolipids have attracted attention for their role in pathophysiology of cardiometabolic diseases. In fact, high plasma ceramides may induce endothelial dysfunction, which is closely correlated with aerobic capacity [13], and promote cell growth arrest, cytoskeleton rearrangements, senescence and death (e.g., activation of caspases), impairment of nitric oxide synthase (eNOS) activity and insulin signaling, increasing vascular permeability, oxidative stress and inflammation [14], thus

contributing to onset and development of atherosclerosis [15,16]. Accordingly, sphingolipids may represent an independent risk determinant for ischemic disease [17]. In particular, Cer(d18:1/16:0) appears to be independently correlated with the presence of more vulnerable coronary plaques in CAD patients [18]. Conversely, the Mediterranean diet shows the potential to reduce the adverse effects of high ceramide levels in the PREDIMED trial (Prevención con Dieta Mediterranea, a prospective case-cohort study), which is a study including nearly 1000 elderly subjects at high cardiovascular risk [19].

Ceramide metabolism also appears responsive to the exercise stimulus. Better cardiopulmonary fitness correlated with low ceramide concentration in elderly coronary artery disease patients has been observed [20], whereas muscle ceramide levels decreased after chronic aerobic exercise [21]. Moreover, experimental data suggests that total content of ceramides decreased in the muscle in trained rats, contributing to the elevation of the glucose uptake observed in skeletal muscles after training [22]. Previous data suggested that 12 weeks of aerobic exercise training in obese or diabetic subjects [23] and 16 weeks of exercise in overweight/obese subjects may reduce sphingolipids (e.g., C18:0, C20:0 and C24:1) [24].

A decrease in ceramide concentrations hints at increased insulin sensitivity, which contributes, at least in part, to the beneficial exercise effects [25]. According to these previous results, we also observed a decrease in total ceramides after the race in trained subjects. Thus, all together these data suggest that sphingolipid species can represent valuable mediators of cardiovascular risk. Studies related to cardiac lipotoxicity showed that the lipotoxic species were primarily driven by ceramides and DAG and not triacylglycerol [26]. Regarding DAG species, Luukkonen et al. showed that DAG (32:1, 34:1, 36:2, 36:3) were significantly increased in subjects with high peripheral insulin resistance versus low peripheral insulin resistance subjects [27] In our study, DAG 36:3 decreased significantly after the race, indicating an improvement of lipotoxicity, attributable to that species after the acute exercise. Experimental data suggest that total content of ceramides decreases in the muscle of trained rats, contributing to the elevation of the glucose uptake observed in skeletal muscles after training [22]. In overweight, obese subjects or type 2 diabetes (T2D), there is a decrease in total ceramides, indicating that endurance training reduces total content of ceramides, likely improving glucose tolerance [22,25,27–29]. However, ceramide metabolism in patients and obese subjects could be dissimilar to that in lean, trained individuals. In fact, the increased lipid metabolism following the physical exercise reduces the substrate availability required for ceramide synthesis (e.g., palmitate, myristate). Moreover, the analysis of the lipid classes in its total assessment may be unsatisfactory, as the specific and complex relationship of different chains of fatty acids in ceramides with chronic and acute exercise demonstrates the complexity of these biological pathways, which require the assessment of increased, decreased or unchanged specific species. Accordingly, our data, for the first time, associate specific molecular species of lipids to exercise. In particular, Cer18:1/16:0 and Cer18:1/18:0 were very low at baseline in athletes and, after the race, the same ceramides increased, indicating a lower consumption of fatty acids contained in these species (palmitic and stearic acid), making these ceramides a non-preferential species for energy purposes. Mardare, Kruger et al. suggest that endurance training in mice is able to reduce long-chain fatty acids ceramides [28]. In particular, this experimental study suggests that the exercise-induced decrease of very long chain fatty acids ceramides (C24:0 and C24:1) may account, almost in part, for the reduced expression of blood inflammatory markers, as well as the increased glucose tolerance. An elevation in long chain fatty acids (C24:0, C24:1, C26:0, C25:0, C22:0) was associated with mitochondrial damage, apoptosis and cell necrosis [29]. It is not completely clear which are the mechanisms able to prevent the elevation in plasma ceramide level correlated with aerobic exercise. In addition to the possibility that lipid utilization during exercise reduces ceramide production by decreasing the availability of substrates required for ceramide synthesis, there are a number of hypothesis-generating ideas [25]; for example, aerobic exercise may accelerate ceramide degradation and clearance by increasing the expression of genes responsible for ceramide

clearance (e.g., acidic and alkaline ceramidase 1 and 3, glucosylceramide synthase and sphingosine kinase 1) [30].

4.3. Inflammatory Levels and Race-Related Trends

IL-6, a pro-inflammatory cytokine, is known to increase after endurance exercise [31]. However, in this setting, IL-6 may have a role as a myokine, and as such, with anti-inflammatory properties, rather than as an inflammatory facilitator [32]. In fact, the observed post-exercise elevations may be in line with exercise-related metabolic IL-6 effects (regulation of glucose homeostasis and fat oxidation) and adaptation to training [33]. Moreover, the release of IL-6 during exercise induces an increase in circulating anti-inflammatory cytokines (e.g., IL-10 and IL-1 receptor antagonist) and decrease of TNFα, or the release of cortisol from the adrenal glands, suggesting that the beneficial effects of exercise (IL-6-mediated) can be expressed, almost in part, through protection against TNFα-induced insulin resistance [34]. These responses, which probably mediate autocrine and paracrine benefits of training, are likely related to training levels, intensity and type of exercise and individual characteristics (e.g., sex and age), and thus may be different in different categories of subjects. Instead, relatively low information is known on the balance between IL-6 and other cytokines and inflammatory biomarkers under such conditions. TNFα did not increase significantly in our population, and this may be the effect of the anti-inflammatory cytokine cascade, which may oppose TNFα increase, giving protection against TNFα-induced damage, as previously observed [35].

CX3CL1 exists in two forms; one form anchors to the membrane, acting as an adhesion molecule, whereas the other form acts as a soluble chemoattractant. CX3CL1 A acts in acute skeletal muscle damage and regeneration through recruitment of macrophages and other immune cells involved in repair and growth of skeletal muscle, influencing the adaptive response to exercise [36,37]. As in our population, previous data suggested an increase of CX3CL1 related to exercise (e.g., cycling) [38]. The variation of circulating CX3CL1 is closely related to changes in muscle gene expression, and as such, might have significance in the adaptive response to exercise [38,39]. Moreover, local CX3CL1 synthesis and expression depend on many factors, including inflammatory cytokines (e.g., TNFα), giving evidence of the relationship that we observed between these two biomarkers [40]. Experimental data suggested that CX3CL1 stimulation of monocytes is associated with a marked increase of TNFα, which is known to stimulate satellite cell proliferation [41,42]. Accordingly, we observed a strong correlation between fractalkine and TNFα, suggesting that this effect could represent an indirect mechanism by which CX3CL1 acts as a mitogen for muscle cells. Moreover, CX3CL1 seems to have beneficial effects in muscle regeneration through a direct effect on myogenic cells [43]. Thus, CX3CL1 likely induces the monocytic and myogenic expression of different factors known to increase in human skeletal muscle after exercise, with a role in the adaptive response following an exercise burst.

Interestingly, in our population, inflammatory biomarkers did not influence the relationships between sphingolipids and exercise, not supporting the existence of a link between inflammation and sphingolipids in half-marathon response, although further studies are needed to verify this possibility. In this context, we also did not observe any relationship between total and species of SM, ceramides and DAG and reactive oxygen species (ROM, a biomarker of oxidative stress) (unshown data) [44].

4.4. Strengths and Limitations

This study has limitations. First, the number of athletes enrolled is not high. However, a strength is that studied athletes were very similar according to training characteristics (day/week of training, Km/week, years of training). Moreover, each subject served as a control for him/herself, increasing the statistical power, and reducing the effects of confounding factors.

Second, insulin was not directly assessed in these subjects. This important issue merits further investigation in future studies to understand the link between species of lipids,

inflammatory markers and insulin resistance (IR) during exercise. However, all our subjects had no history of IR and type 2 diabetes (T2D) and they showed normal body mass index; therefore, this relationship could be negligible. Nonetheless, it will be interesting to assess exercise effects on lipid-related biomarkers in IR, T2D or obese subjects in future studies.

5. Conclusions

IL-6 and CX3CL1 transiently increased soon after the half-marathon. As they are promptly reversible, these changes might represent a physiological response to acute exercise rather than a damage-related response. The decrease in plasma ceramide concentration observed after the race and the lack of their relationship with inflammatory mediators suggested the involvement of lipids in exercise adaptation, as well as their possible role in mechanisms underlying beneficial effects of regular physical activity on disease prevention, especially regarding cardiometabolic disease. This opens up a new area of investigation for future research to establish whether the measurement of specific plasma ceramides could provide new biomarkers useful to assess exercise adaptation and to evaluate specific exercise interventions in different categories of healthy subjects and patients.

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References

1. Knechtle, B.; Nikolaidis, P.T.; Zingg, M.A.; Rosemann, T.; Rüst, C.A. Half-marathoners are younger and slower than marathoners. *SpringerPlus* **2016**, *5*, 1–16. [CrossRef]
2. Nieman, D.C.; Henson, D.A.; Smith, L.L.; Utter, A.C.; Vinci, D.M.; Davis, J.M.; Kaminsky, D.E.; Shute, M. Cytokine changes after a marathon race. *J. Appl. Physiol.* **2001**, *91*, 109–114. [CrossRef]
3. Nikolova-Karakashian, M.N.; Reid, M.B. Sphingolipid Metabolism, Oxidant Signaling, and Contractile Function of Skeletal Muscle. *Antioxidants Redox Signal.* **2011**, *15*, 2501–2517. [CrossRef] [PubMed]
4. Nishi, H.; Higashihara, T.; Inagi, R. Lipotoxicity in Kidney, Heart, and Skeletal Muscle Dysfunction. *Nutrients* **2019**, *11*, 1664. [CrossRef]
5. Portt, L.; Norman, G.; Clapp, C.; Greenwood, M.; Greenwood, M.T. Anti-apoptosis and cell survival: A review. *Biochim. Biophys. Acta (BBA) Bioenerg.* **2011**, *1813*, 238–259. [CrossRef]
6. Chakraborty, M.; Jiang, X.-C. Sphingomyelin and Its Role in Cellular Signaling. *Chem. Biol. Pteridines Folates* **2013**, *991*, 1–14. [CrossRef]
7. Conroy, M.J.; Maher, S.G.; Melo, A.M.; Doyle, S.L.; Foley, E.; Reynolds, J.V.; Long, A.; Lysaght, J. Identifying a Novel Role for Fractalkine (CX3CL1) in Memory CD8+ T Cell Accumulation in the Omentum of Obesity-Associated Cancer Patients. *Front. Immunol.* **2018**, *9*, 1867. [CrossRef] [PubMed]
8. Gunga, H.-C.; Kirsch, K.; Behn, C.; Koralewski, E.; Davila, E.H.; Estrada, M.I.; Johannes, B.; Wittels, P.; Jelkmann, W. Vascular endothelial growth factor in exercising humans under different environmental conditions. *Graefe's Arch. Clin. Exp. Ophthalmol.* **1999**, *79*, 484–490. [CrossRef] [PubMed]

9. Fischer, C.P. Interleukin-6 in acute exercise and training: What is the biological relevance? *Exerc. Immunol. Rev.* **2006**, *12*, 41.
10. Idriss, H.T.; Naismith, J.H. TNFα and the TNF receptor superfamily: Structure-function relationship(s). *Microsc. Res. Tech.* **2000**, *50*, 184–195. [CrossRef]
11. Hackl, M.T.; Fürnsinn, C.; Schuh, C.M.; Krssak, M.; Carli, F.; Guerra, S.; Freudenthaler, A.; Baumgartner-Parzer, S.; Helbich, T.H.; Luger, A.; et al. Brain leptin reduces liver lipids by increasing hepatic triglyceride secretion and lowering lipogenesis. *Nat. Commun.* **2019**, *10*, 1–13. [CrossRef]
12. Lewis, G.D.; Farrell, L.; Wood, M.J.; Martinovic, M.; Arany, Z.; Rowe, G.C.; Souza, A.; Cheng, S.; McCabe, E.L.; Yang, E.; et al. Metabolic Signatures of Exercise in Human Plasma. *Sci. Transl. Med.* **2010**, *2*, 33–37. [CrossRef] [PubMed]
13. Montero, D. The association of cardiorespiratory fitness with endothelial or smooth muscle vasodilator function. *Eur. J. Prev. Cardiol.* **2015**, *22*, 1200–1211. [CrossRef] [PubMed]
14. Haus, J.M.; Kashyap, S.R.; Kasumov, T.; Zhang, R.; Kelly, K.R.; DeFronzo, R.A.; Kirwan, J.P. Plasma Ceramides Are Elevated in Obese Subjects With Type 2 Diabetes and Correlate With the Severity of Insulin Resistance. *Diabetes* **2008**, *58*, 337–343. [CrossRef]
15. Ichi, I.; Nakahara, K.; Miyashita, Y.; Hidaka, A.; Kutsukake, S.; Inoue, K.; Maruyama, T.; Miwa, Y.; Harada-Shiba, M.; Tsushima, M.; et al. Association of ceramides in human plasma with risk factors of atherosclerosis. *Lipids* **2006**, *41*, 859–863. [CrossRef]
16. Bismuth, J.; Lin, P.; Yao, Q.; Chen, C. Ceramide: A common pathway for atherosclerosis? *Atherosclerosis* **2008**, *196*, 497–504. [CrossRef]
17. Jiang, X.-C.; Paultre, F.; Pearson, T.A.; Reed, R.G.; Francis, C.K.; Lin, M.; Berglund, L.; Tall, A.R. Plasma Sphingomyelin Level as a Risk Factor for Coronary Artery Disease. *Arter. Thromb. Vasc. Biol.* **2000**, *20*, 2614–2618. [CrossRef]
18. Anroedh, S.S.; Hilvo, M.; Akkerhuis, K.M.; Kauhanen, D.; Koistinen, K.; Oemrawsingh, R.; Serruys, P.; van Geuns, R.-J.; Boersma, E.; Laaksonen, R.; et al. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J. Lipid Res.* **2018**, *59*, 1729–1737. [CrossRef]
19. Wang, D.D.; Toledo, E.; Hruby, A.; Rosner, B.A.; Willett, W.C.; Sun, Q.; Razquin, C.; Zheng, Y.; Ruiz-Canela, M.; Guasch-Ferré, M.; et al. Plasma Ceramides, Mediterranean Diet, and Incident Cardiovascular Disease in the PREDIMED Trial (Prevención con Dieta Mediterránea). *Circulation* **2017**, *135*, 2028–2040. [CrossRef]
20. Saleem, M.; Herrmann, N.; Dinoff, A.; Marzolini, S.; Mielke, M.M.; Andreazza, A.; I Oh, P.; Venkata, S.L.V.; Haughey, N.J.; Lanctôt, K.L. Association Between Sphingolipids and Cardiopulmonary Fitness in Coronary Artery Disease Patients Undertaking Cardiac Rehabilitation. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2020**, *75*, 671–679. [CrossRef]
21. Bruce, C.R.; Thrush, A.B.; Mertz, V.A.; Bezaire, V.; Chabowski, A.; Heigenhauser, G.J.F.; Dyck, D.J. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am. J. Physiol. Metab.* **2006**, *291*, E99–E107. [CrossRef]
22. Dobrzyń, A.; Zendzian-Piotrowska, M.; Górski, J. Effect of endurance training on the sphingomyelin-signalling pathway activity in the skeletal muscles of the rat. *J. Physiol. Pharmacol. Off. J. Pol. Physiol. Soc.* **2004**, *55*, 305–313.
23. Kasumov, T.; Solomon, T.P.; Hwang, C.; Huang, H.; Haus, J.M.; Zhang, R.; Kirwan, J.P. Improved insulin sensitivity after exercise training is linked to reduced plasma C14:0 ceramide in obesity and type 2 diabetes. *Obesity* **2015**, *23*, 1414–1421. [CrossRef] [PubMed]
24. Dubé, J.J.; Amati, F.; Toledo, F.G.S.; Stefanovic-Racic, M.; Rossi, A.; Coen, P.; Goodpaster, B.H. Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. *Diabetologia* **2011**, *54*, 1147–1156. [CrossRef]
25. Scherer, P.E.; Hill, J.A. Obesity, Diabetes, and Cardiovascular Diseases. *Circ. Res.* **2016**, *118*, 1703–1705. [CrossRef] [PubMed]
26. Luukkonen, P.K.; Zhou, Y.; Sädevirta, S.; Leivonen, M.; Arola, J.; Orešič, M.; Hyötyläinen, T.; Yki-Järvinen, H. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. *J. Hepatol.* **2016**, *64*, 1167–1175. [CrossRef] [PubMed]
27. Dubé, J.J.; Amati, F.; Stefanovic-Racic, M.; Toledo, F.G.S.; Sauers, S.E.; Goodpaster, B.H. Exercise-induced alterations in intramyocellular lipids and insulin resistance: The athlete's paradox revisited. *Am. J. Physiol. Metab.* **2008**, *294*, E882–E888. [CrossRef] [PubMed]
28. Bergman, B.C.; Brozinick, J.T.; Strauss, A.; Bacon, S.; Kerege, A.; Bui, H.H.; Sanders, P.; Siddall, P.; Kuo, M.S.; Perreault, L. Serum sphingolipids: Relationships to insulin sensitivity and changes with exercise in humans. *Am. J. Physiol. Metab.* **2015**, *309*, E398–E408. [CrossRef] [PubMed]
29. Mardare, C.; Krüger, K.; Liebisch, G.; Seimetz, M.; Couturier, A.; Ringseis, R.; Wilhelm, J.; Weissmann, N.; Eder, K.; Mooren, F.-C. Endurance and Resistance Training Affect High Fat Diet-Induced Increase of Ceramides, Inflammasome Expression, and Systemic Inflammation in Mice. *J. Diabetes Res.* **2015**, *2016*, 1–13. [CrossRef] [PubMed]
30. Hartmann, D.; Lucks, J.; Fuchs, S.; Schiffmann, S.; Schreiber, Y.; Ferreirós, N.; Merkens, J.; Marschalek, R.; Geisslinger, G.; Grösch, S. Long chain ceramides and very long chain ceramides have opposite effects on human breast and colon cancer cell growth. *Int. J. Biochem. Cell Biol.* **2012**, *44*, 620–628. [CrossRef]
31. Cappuccilli, M.; Mosconi, G.; Roi, G.; De Fabritiis, M.; Totti, V.; Merni, F.; Trerotola, M.; Marchetti, A.; La Manna, G.; Costa, A.N. Inflammatory and Adipose Response in Solid Organ Transplant Recipients After a Marathon Cycling Race. *Transplant. Proc.* **2016**, *48*, 408–414. [CrossRef] [PubMed]

32. Gill, S.K.; Teixeira, A.; Rama, L.; Prestes, J.; Rosado, F.; Hankey, J.; Scheer, V.; Hemmings, K.; Ansley-Robson, P.; Costa, R.J.S. Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. *Exerc. Immunol. Rev.* **2015**, *21*, 114–128.
33. Pedersen, B.K.; Åkerström, T.C.A.; Nielsen, A.R.; Fischer, C.P. Role of myokines in exercise and metabolism. *J. Appl. Physiol.* **2007**, *103*, 1093–1098. [CrossRef]
34. Ostrowski, K.; Rohde, T.; Asp, S.; Schjerling, P.; Pedersen, B.K. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J. Physiol.* **1999**, *515*, 287–291. [CrossRef]
35. Febbraio, M.A.; Pedersen, B.K. Muscle-derived interleukin-6: Mechanisms for activation and possible biological roles. *FASEB J.* **2002**, *16*, 1335–1347. [CrossRef]
36. Chapman, G.A.; Moores, K.E.; Gohil, J.; Berkhout, T.A.; Patel, L.; Green, P.; Macphee, C.H.; Stewart, B.R. The role of fractalkine in the recruitment of monocytes to the endothelium. *Eur. J. Pharmacol.* **2000**, *392*, 189–195. [CrossRef]
37. Goda, S.; Imai, T.; Yoshie, O.; Yoneda, O.; Inoue, H.; Nagano, Y.; Okazaki, T.; Imai, H.; Bloom, E.T.; Domae, N.; et al. CX3C-Chemokine, Fractalkine-Enhanced Adhesion of THP-1 Cells to Endothelial Cells Through Integrin-Dependent and -Independent Mechanisms. *J. Immunol.* **2000**, *164*, 4313–4320. [CrossRef]
38. Catoire, M.; Mensink, M.; Kalkhoven, E.; Schrauwen, P.; Kersten, S. Identification of human exercise-induced myokines using secretome analysis. *Physiol. Genom.* **2014**, *46*, 256–267. [CrossRef] [PubMed]
39. Pillon, N.J.; Bilan, P.J.; Fink, L.N.; Klip, A. Cross-talk between skeletal muscle and immune cells: Muscle-derived mediators and metabolic implications. *Am. J. Physiol. Metab.* **2013**, *304*, E453–E465. [CrossRef] [PubMed]
40. Wojdasiewicz, P.; Turczyn, P.; Dobies-Krzesniak, B.; Frasunska, J.; Tarnacka, B. Role of CX3CL1/CX3CR1 Signaling Axis Activity in Osteoporosis. *Mediat. Inflamm.* **2019**, *2019*, 1–9. [CrossRef]
41. Strömberg, A.; Olsson, K.; Dijksterhuis, J.P.; Rullman, E.; Schulte, G.; Gustafsson, T. CX3CL1—A macrophage chemoattractant induced by a single bout of exercise in human skeletal muscle. *Am. J. Physiol. Integr. Comp. Physiol.* **2016**, *310*, R297–R304. [CrossRef] [PubMed]
42. Li, Y.-P. TNF-α is a mitogen in skeletal muscle. *Am. J. Physiol. Physiol.* **2003**, *285*, C370–C376. [CrossRef] [PubMed]
43. Sonnet, C.; Lafuste, P.; Arnold, L.; Brigitte, M.; Poron, F.; Authier, F.J.; Chrétien, F.; Gherardi, R.K.; Chazaud, B. Human macrophages rescue myoblasts and myotubes from apoptosis through a set of adhesion molecular systems. *J. Cell Sci.* **2006**, *119*, 2497–2507. [CrossRef]
44. Vassalle, C.; Del Turco, S.; Sabatino, L.; Basta, G.; Maltinti, M.; Sbrana, F.; Ndreu, R.; Mastorci, F.; Pingitore, A. New inflammatory and oxidative stress-based biomarker changes in response to a half-marathon in recreational athletes. *J. Sports Med. Phys. Fit.* **2020**, *60*. [CrossRef]