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Fatty acid metabolism in an oribatid mite: de novo biosynthesis and the effect of starvation

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Abstract
The fatty acid (FA) composition of lipids in animals is influenced by factors such as species, life stage, availability and type of food, as well as the ability to synthesize certain FAs de novo. We investigated the effect of starvation on the neutral lipid (NLFA) and phospholipid (PLFA) fatty acid patterns of the oribatid mite *Archegozetes longisetosus* Aoki. Furthermore, we performed stable-isotope labeled precursors feeding experiments under axenic conditions to delineate de novo FA synthesis by profiling ^{13}C and deuterium incorporation via single-ion monitoring. Starvation of mites resulted in a decline in the total amount of NLFAs and significantly changed the fatty acid patterns, indicating that NLFAs were metabolized selectively. Biochemical tracer experiments confirmed that oribatid mites, like other animals, can produce stearic (18:0) and oleic acid (18:1ω9) de novo. Mass spectrometric data also revealed that they appear to synthesize linoleic acid [18:2ω6,9 = (9Z,12Z)-octadeca-9,12-dienoic acid]—an ability restricted only to a few arthropod taxa, including astigmatid mites. The physiological and biosynthesis processes revealed here are crucial to understand the potential biomarker function of fatty acids—especially 18:2ω6,9—in oribatid mites and their applicability in soil animal food web studies.

Keywords Neutral lipid fatty acids · Starvation · Stable-isotope labeling · Soil animal · Biochemistry

Introduction

Oribatid mites (Arachnida, Actinotrichida, Oribatida) are a species-rich group of mostly soil-dwelling arthropods comprising approximately 11,000 described species (Schatz et al. 2011). The ecosystem functions of these ubiquitously distributed animals are primarily

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related to decomposing processes in soils as they foster soil structure and fertility by contributing to elemental cycles (Behan-Pelletier 1997; Bünemann et al. 2018; Seastedt 1984). In general, oribatid mites can be classified as primary and secondary decomposers, lichen feeders, and scavengers (Heidemann et al. 2011; Maraun et al. 2011). As for other soil animals, direct assessment of resource preferences and classification of feeding niches is difficult as observations are hampered by the enigmatic nature of soils. Therefore, methods like stable-isotope analysis (Pollierer et al. 2019; Potapov et al. 2019), molecular gut-content analysis (Harper et al. 2005; King et al. 2008), or the biochemical trace analysis of marker fatty acids from neutral- and phospholipids (NLFAs = neutral lipid fatty acids, PLFAs = phospholipid fatty acids) (Ruess and Chamberlain 2010) have become a major tool to study the translocation of elements, the flow of energy as well as the structure of complex soil food webs. To reliably use fatty acids as trophic biomarkers in a certain taxon it is, however, necessary to understand their trophic transfer from the food to the animal, as well as the basic metabolism (e.g. de novo biosynthesis) and physiological modifications (e.g., feeding situation, environmental temperature, or life-stage) within the animal (Ruess and Chamberlain 2010). Whereas many of these physiological processes are well understood in nematodes and collembolans (Haubert et al. 2004, 2006, 2008; Menzel et al. 2017, 2018), they are more or less unexplored in other important soil animal taxa like mites or litter-inhabiting ants (Rosumek et al. 2017, 2018).

Focusing on oribatid mite fatty acids, only three studies exist: one tested the use of NLFAs as food-web biomarkers in a model system (Brückner et al. 2017), a second applied NLFAs to investigate variation in trophic niches of oribatid mites in a temperate forest (Maraun et al. 2020), whereas a third used fatty acids as tracer molecules in a carbon-flux experiment (Pollierer et al. 2012). So far, no biochemical study about the basic fatty acid metabolism of oribatid mites exists. Some older morphological investigations and life-history experiments suggest that oribatid mites have well-developed fat bodies but may lack the ability to store large amounts of metabolic energy for a long time (Wallwork 1979). Thus, feeding restriction should evoke a relatively quick physiological response and the amount of stored lipids ought to decrease rapidly. Furthermore, the general biochemistry of fatty acid synthesis in oribatid mites remains unknown, yet one of our former trophic transfer experiments (Brückner et al. 2017) suggested that oribatid mites are most likely producing basic fatty acids like stearic (18:0) or oleic acid (18:1ω9) de novo and might also be able to synthesize linoleic acid [18:2ω6,9= (9*Z*,12*Z*)-octadeca-9,12-dienoic acid]. The latter hypothesis is also underpinned by two studies on astigmatid mites (Actinotrichida, Astigmata), which may be phylogenetically nested within oribatid mites (Norton 1994), that demonstrated the biosynthesis of 18:2ω6,9—potentially *via* a Δ-12 desaturase enzyme (Aboshi et al. 2013; Shimizu et al. 2014). Yet, both studies could not rule out the possibility that bacteria might play a role in the biosynthesis of 18:2ω6,9, as they were not performed in axenic conditions.

In this study, we exploit the oribatid model species *Archegozetes longisetosus* Aoki (Actinotrichida, Oribatida, Trhypochthoniidae) (i) to explore the effects of feeding situation on oribatid mite NLFAs by prolonged starving, and (ii) study the in vivo biosynthesis of C_{18}-fatty acids (18:0, 18:1ω9, 18:2ω6,9) biochemically by using stable isotope-labeling and single-ion mass spectrometry. We expected that (i) similar to other soil animals (e.g., Canavoso et al. 1998; Haubert et al. 2004), *A. longisetosus*' total NLFA amounts would decline steadily upon starvation, but that the overall NLFA composition does not change significantly; and (ii) that *A. longisetosus* would be able to synthesize 18:2ω6,9 de novo given that phylogenetically closely related astigmatid mites are able to do so (Aboshi et al. 2013; Shimizu et al. 2014).

Materials and methods

Model species and starvation experiment

The 'ran'-strain of the parthenogenetic oribatid mite *A. longisetosus* was used as experimental model species in this study (Heethoff et al. 2007). *Archegozetes longisetosus* is an opportunistic feeder and can thus be cultured on various powdered foods which can easily be manipulated (Brückner et al. 2018).

For the starvation experiment about 1500 specimens were removed from different stock cultures fed with wheat grass powder (Naturya, Bath, UK). Only individuals with already emptied alimentary tract (visual inspection) were removed from the stock cultures and haphazardly distributed across the four experimental vessels (approx. 350–400 individuals per box). After that, mites were not fed, and only water was resupplied 3 × a week. At days 0, 3, 6, 9, 16, 23 and 30 we collected 35 specimens per experimental vessel and froze them at −28 °C until further analysis.

Labeling experiment

To investigate the biosynthesis of C_{18}-fatty acids we performed a feeding experiment with stable isotope-labeled D-glucose (Shimizu et al. 2014) under sterile conditions. Collected eggs of *A. longisetosus* were surface-washed with sodium hypochlorite solution (3% w/v), ethanol (70% v/v) and sterilized water for 5, 15 and 30 s, respectively. Afterwards, eggs were transferred to sterile Petri dishes (45 mm diameter) lined with sterile, analytical filter paper (Hartenstein, Würzburg, Germany). The cultures were maintained in a laminar-flow closet at 28 °C and 90% relative humidity and supplied with sterilized water and approx. 1 mg food 3 × each week, old food was discharged. For the control group we fed pure sterilized (10 h at 160 °C) wheat grass, whereas treatment group specimens were fed with a mixture of sterilized wheat grass powder with amoxicillin, streptomycin as well as tetracycline (ca. 3.3% w/w for each) and 25% (w/w) of the stable isotope-labeled precursor [$^{13}C_6$, d_7] D-glucose. The cultures were maintained for one generation (ca. 50 days) and one week after adult eclosion mites were harvested and again frozen at −28 °C. Antibiotics were purchased from Sigma-Aldrich (St. Louis, MO, USA) and glucose from Cambridge Isotope Laboratories (Tewksbury, MA, USA).

Fatty acid analyses

For both experiments (starvation and labeling) total lipids were extracted from each sample using 1 ml of chloroform:methanol, 2:1 (v/v) according to Folch et al. (1957) over a period of 24 h. Mites from the starvation experiment were dried over a period of 3 days after the extraction weighted to the nearest of 0.1 µg using a microbalance (XS3DU, Mettler Toledo, Columbus, OH, USA). Lipid extracts were purified and separated according to Frostegård et al. (1991); in short: SiOH-columns (Chromabond® SiOH, Macherey-Nagel, Düren, Germany) were washed and conditioned with 6 ml hexane. Subsequently, samples were applied to the column and elution of neutral lipids was accomplished with 4 ml chloroform. Glycolipids were eluted with 5 ml acetone, followed by phospholipids which were washed of the column with 4 ml methanol. Afterwards the chloroform (containing neutral lipids) and methanol (containing the phospholipids) fractions were evaporated to dryness under gentle nitrogen gas flow and residuals were re-dissolved in 300 µl dichloromethane:methanol, 2:1

(v/v), 100 µl of this extract was transferred to new glass vials with a conical inlet. Additionally, 20 µl of internal standard ($C_{19:0}$ in methanol; 220 ng/µl) were added before the samples were evaporated to dryness again. Subsequently, samples were derivatized to fatty acid methyl esters (FAMEs) with 20 µl TMSH (trimethylsulfonium hydroxide; 0.25 m in MeOH from Fluka, Sigma-Aldrich) in a one-step reaction.

FAMEs of neutral lipid fatty acids (NLFAs) and phospholipid fatty acids (PLFAs) were analyzed with a QP2010 Ultra gas chromatography–mass spectrometry system from Shimadzu (Kyoto, Japan) equipped with a ZB-5MS capillary column (0.25 mm × 30 m, 0.25 µm film thickness) from Phenomenex (Torrance, CA, USA). Hydrogen was used as carrier gas with a flow rate of 3.00 ml/min, with splitless injection (300 °C) and a temperature ramp was set to increase from 60 °C (1 min hold), to 150 °C with 15 °C/min, to 260 °C with a heating-rate of 3 °C/min, followed by an increase to 320 °C with 10 °C/min (isothermal hold for 10 min). Electron ionization mass spectra were recorded at 70 eV either (i) in single-ion mode targeting characteristic ions to trace incorporation of ^{13}C and d, or (ii) from $m/z = 40–450$ for NLFA/PLFA quantification. The ion source of the mass spectrometer and the transfer line were kept at 250 °C. FAMEs were identified by comparison with the Supelco® 37 Component FAME Mix standard (Sigma-Aldrich).

Data processing and statistics

The amounts of NLFA and PLFA methyl esters were standardized by the dry weight of animals, whereas the compositional data of the fatty acids were ordinated as Bray–Curtis similarity and non-metric multidimensional scaling (NMDS). As we were interested in the changes of NLFAs that are most likely de novo produced by the mites (Shimizu et al. 2014) and not derived from food, we excluded the clearly dietary derived NLFAs (Brückner et al. 2017). The initial NLFA/PLFA amounts of the mites were compared using a paired t-test. The subsequent measurements of the total NLFA content, the lean dry weight, the C_{16}:C18-ratio and the unsaturation index (UI, calculated as $UI = [(C:1 \times 1) + (C:2 \times 2)]/100$, where C:1 and C:2 are the proportion of fatty acids with 1 and 2 double bonds, respectively) over time were analyzed using repeated measure ANOVAs to account for the resampling from the four experimental vessels. The total NLFA amount was log-transformed to ensure the normality of residuals and homogeneity of variance. All these analyses were performed with PAST 3 (Hammer et al. 2001). For the multivariate data we performed a PERMDISP to check for homogeneity of multivariate dispersions ($F_{6,21} = 1.6$, $p = 0.35$) and PERMANOVA to test whether the relative composition of storage NLFAs also changed with prolonged starvation. To account for vessel resampling, we used their ID as random factor. Multivariate analyses were performed with Primer 7, v.7.0.12 (Primer-E, Ivybridge, UK) with the add-on PERMANOVA + 1.

Results

Fatty acid analysis and the effect of starvation

The amount of neutral lipid fatty acids (mean ± SD = 41.20 ± 6.83 µg/mg) of non-starved, ad libitum fed oribatid mites was about 70 × higher than the amount of phospholipid fatty acids (0.58 ± 0.27 µg/mg; paired t-test: n = 08, t = 10.3; p = 0.002; Fig. 1a). Starvation led to declining NLFA amounts over time (repeated measure ANOVA: $F_{6,21} = 86.9$, $p < 0.0001$;

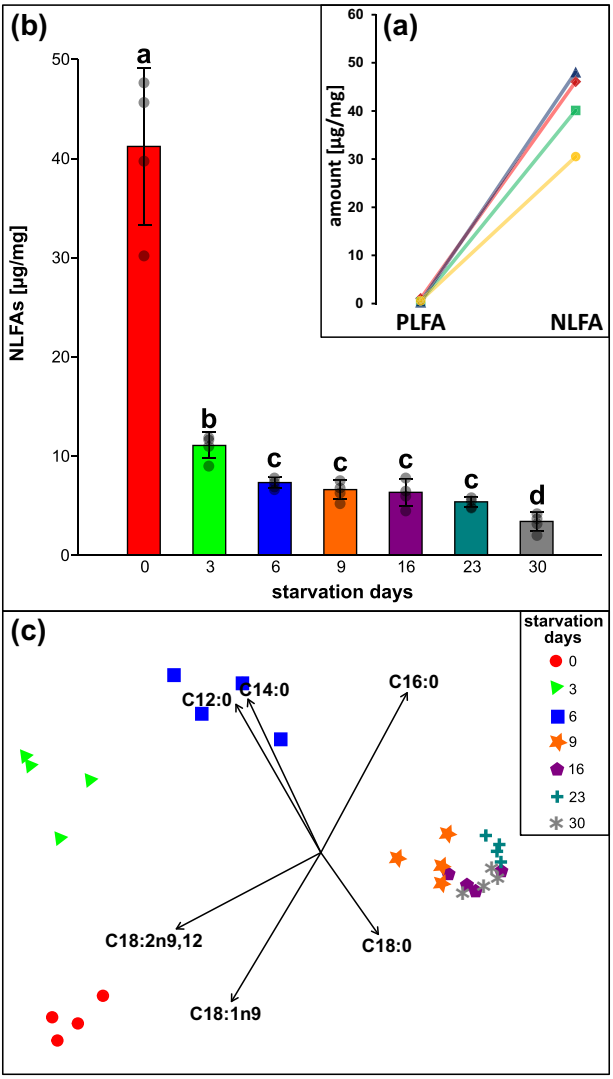

Fig. 1 Biochemical analysis of oribatid mite neutral lipid fatty acids (NLFAs) and the effect of starvation. Comparison of membrane phospholipid fatty acid (PLFAs) and NLFA amounts (in µg/mg sample) from the same mite samples, colors and line denote the paired samples (**a**). The effect of prolonged starvation on the total amount of NLFAs (**b**) and the composition of NLFAs (**c**) of *Archegozetes longisetosus*. Color code refers to starvation days. Same letters indicate non-significant categories (Tukey's HSD after repeated-measured ANOVA, **b**). 2D-stress of the NMDS = 0.06, individual fatty acids were mapped onto the NMDS ordination plot as vectors (**c**). (Color figure online)

Fig. 1b; Table 1) and a change in relative composition of storage NLFAs (PERMANOVA: pseudo$F_{6,21}$ = 54.3, $p < 0.0001$; Fig. 1c). In contrast, the lean dry weight (i.e., dried animals after fat extraction; 364.71 ± 100.22 µg/sample) did not change over the starvation period (repeated measure ANOVA: $F_{6,21}$ = 0.2, $p = 0.63$). The C_{16}:C_{18}-ratio as well as the unsaturation index (UI) changed over time when mites were starved, resulting in a higher

Table 1 Neutral lipid fatty acid (NLFA) composition as well as the mean (±SD) total amount of NLFAs, C_{16}:C_{18}-ratio and the unsaturation index (UI) of *Archegozetes longisetosus* after 0–30 days of starvation

	Starvation period (days)						
	0	3	6	9	16	23	30
Saturated							
12:0	0.14 ± 0.06	1.69 ± 0.30	0.93 ± 0.35	0.11 ± 0.05	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.00
14:0	0.24 ± 0.06	1.63 ± 0.87	1.43 ± 0.73	0.07 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	0.04 ± 0.01
16:0	15.25 ± 3	45.26 ± 1.95	55.37 ± 4.5	52.02 ± 2.81	54.1 ± 3.51	63.14 ± 1.91	54.52 ± 3.34
18:0	37.69 ± 3.43	38.84 ± 1.91	33.5 ± 2.54	45.5 ± 1.8	44.52 ± 3.12	36.31 ± 1.94	44.43 ± 2.86
Unsaturated							
18:1ω9	43.93 ± 5.9	8.79 ± 1.99	8.76 ± 2.96	2.31 ± 1.39	1.29 ± 0.38	0.44 ± 0.09	0.98 ± 0.52
18:2ω6,9	2.77 ± 0.33	3.79 ± 1.27	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
NLFAs (µg/mg)	41.2 ± 6.83	11.06 ± 1.14	7.32 ± 0.45	6.61 ± 0.84	6.34 ± 1.19	5.38 ± 0.44	3.4 ± 0.81
C_{16}:C_{18}	0.18 ± 0.04a	0.88 ± 0.09b	1.33 ± 0.23c	1.1 ± 0.12b	1.19 ± 0.17bc	1.73 ± 0.14c	1.21 ± 0.16bc
UI	0.49 ± 0.06a	0.16 ± 0.02b	0.09 ± 0.03c	0.02 ± 0.01d	0.01 ± < 0.01e	< 0.01 ± < 0.01e	0.01 ± 0.01e

Means within a row followed by different letters are significantly different (repeated-measured ANOVA followed by Tukey's HSD: $p < 0.05$)

C_{16}:C_{18}-ratio and a lower UI (C_{16}:C_{18}-ratio: repeated measure ANOVA: $F_{6,21} = 30.8$, $p < 0.001$; UI: repeated measure ANOVA: $F_{6,21} = 52.9$, $p < 0.0001$; for both see Table 1).

Incorporation of labeled D-glucose into C_{18}-NLFAs

The mass spectrum of 18:0-methyl ester from control group mites showed a M^+ ion at $m/z = 298$ (Fig. 2) and the intensity of the isotope ion ($M^+ + 1$ at $m/z = 299$) compared to M^+ ($m/z = 298$) was 23.3%. Feeding with labeled D-glucose increased this value to 28.2% and the intensity of further ions (Fig. 2) increased, as well ($m/z = 300$, 8.6%; $m/z = 301$, 4.3%; $m/z = 302$, 2.6%; $m/z = 303$, 1.9%). The mass spectra of 18:1ω9- and 18:2ω6,9-methyl esters showed a similar pattern (Fig. 2). In the control group the $M^+ + 1$ ions had intensities of 22.0% (18:1ω9) and 22.5% (18:2ω6,9) compared to the M^+, whereas these values were increased in the labeled D-glucose fed mites (30.9 and 31.6%, respectively). Again, also the further ions showed a *m/z*-intensity increase for the $M^+ + 2$ to $M^+ + 5$ ions of both fatty acid methyl esters (Fig. 2).

Discussion

Lipids play a pivotal role in every organism: storage triacyclglycerols are essential reserves of metabolic energy, whereas phospholipids are the major constituents of biological membranes. Like for most other terrestrial arthropods, amounts of storage fatty acids (NLFAs) were much higher compared to membrane bound fatty acids (PLFAs; Fig. 1). For instance, in collembolans about 5% of the total fatty acids were PLFAs (Haubert et al. 2004), whereas PLFAs only compromised ca. 2% of the fatty acids isolated from *A.*

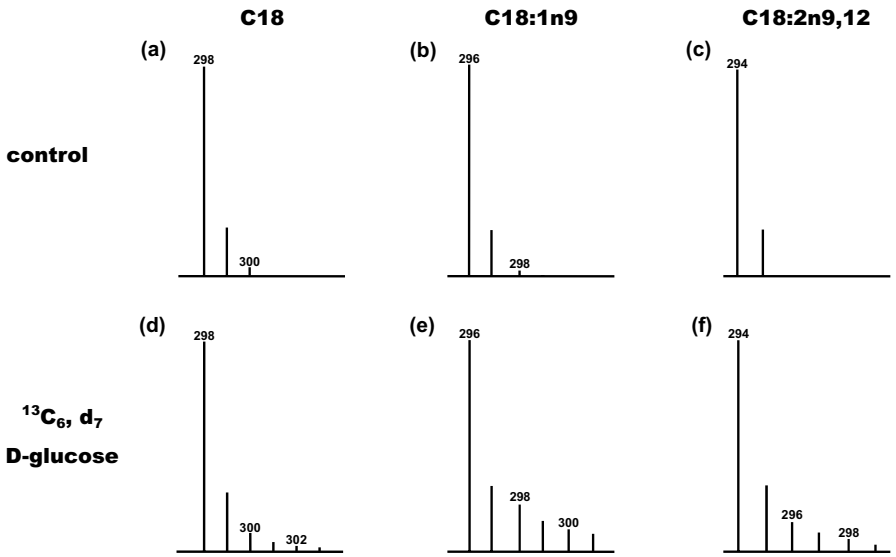

Fig. 2 Representative mass spectra of control (unlabeled) and labeled C_{18} fatty acids (**a** + **d** = stearic acid: 18:0; **b** + **e** = oleic acid: 18:1ω9; **c** + **f** = linoleic acid: 18:2ω6,9) recorded in single-ion mode. Mites fed with a mixture of labeled [$^{13}C_6$, d_7] D-glucose and antibiotics show enriched ions

longisetosus. As excepted and found in other terrestrial arthropods (Haubert et al. 2004; Hilligsøe and Holmstrup 2003; Lavy et al. 1997; McCue 2012), the storage lipid fatty acid amount declined shortly after the mites became food restricted (Fig. 1). The lower amounts of NLFAs were reached faster than in other investigated soil animals, as NLFA amounts already decline after three days of starvation. This quick physiological response seems to confirm the hypothesis by Wallwork (1979), i.e., oribatid mites appear to lack the ability to store large amounts of metabolic energy for a long time. On the other hand, this also suggests that oribatid mites should be able to re-synthesize fatty acids for their lipid storage quickly and may have a rather fast fatty acid anabolism and catabolism turnaround time (Haubert 2006). This idea is supported by Aboshi et al. (2013) and Shimizu et al. (2014) who showed that deuterium labeled fatty acids and ^{13}C from glucose were readily measurable in phospholipids and storage lipids of astigmatid mites after only 3 days or 3 weeks, respectively. The body mass of collembolans drastically declines during periods of starvation (Hilligsøe and Holmstrup 2003; Lavy et al. 1997), yet the lean dry weights of the mites were not influenced by food deprivation. This could indicate that there was no metabolic turnover of structural body components such as proteins or chitin, but also low levels of physiological stress related to non-fatty acid metabolic pathways.

Lipids do not have a fixed composition, the selection of fatty acids is rather influenced by diet and general nourishment situation, but also by age or general physiological stress (Howard et al. 1993; Stanley-Samuelson et al. 1988). The abundance of individual NLFAs is determined by the de novo synthesis rate, fatty acid mobilization and catabolic use. Hence, NLFA composition might serve as a proxy to predict the interplay and biochemical connection of certain fatty acids. Similar experiments with springtails (Haubert et al. 2004) and assassin bugs (Canavoso et al. 1998, 2003) demonstrated that the complex blend of NLFAs was indiscriminately metabolized when the animals were starved and no noticeable changes of NLFA composition was detected. In contrast, our experiments with the oribatid mite *A. longisetosus* (Fig. 1; Table 1) showed that the relative abundance of 18:2ω6,9 as well as 18:1ω9 and to a lesser extend also the shorter-chained fatty acids (12:0 and 14:0) declined. As a consequence, the unsaturation index of storage NLFAs dropped (Table 1). Although selective mobilization and metabolization of fatty acids appears to be uncommon in terrestrial arthropods (Canavoso et al. 1998, 2003, 2004; Haubert et al. 2004), this mechanism is well known from the fat metabolism of vertebrates, including humans (Hagenfeldt and Wahren 1968; McCue 2012). During starvation, short-chained and unsaturated NLFAs are preferentially oxidized, whereas other fatty acids are selectively retained (Price et al. 2008; Raclot 2003; Raclot and Groscolas 1993). The observed shift in relative NLFA composition during times of food deprivation (Fig. 1) seems to be the rule for most animals, whereas the other data on soil arthropods are the exception (Haubert et al. 2004). Interestingly, the co-decline of 18:2ω6,9 and 18:1ω9 suggests a biosynthetic connection of both fatty acids.

Mass spectrometric data confirmed that *A. longisetosus* synthesizes 18:0 and 18:1ω9 de novo (Fig. 2). The ability to produce these two fatty acids is commonly shared among most organisms (i.e., bacteria, fungi, plants and animals; Smith 1994) and it is not surprising that oribatid mites also share this ability. The capability to synthesize 18:2ω6,9 was thought to be restricted to bacteria, fungi and plants for a long time. In the last 35 years, however, it became clear that animals can synthesize 18:2ω6,9 de novo, including nematodes (Wallis et al. 2002), snails (Weinert et al. 1993), protozoans (Sayanova et al. 2006) and several arthropods (see reviewed by Malcicka et al. 2018), including astigmatid mites (Aboshi et al. 2013; Shimizu et al. 2014). Our mass spectrometric data (Fig. 2) confirmed that *β-polycarbonyl* units (i.e., acetyl-CoA and manoyl-CoA) produced from labeled

glucose via glycolysis were incorporated in 18:2ω6,9, suggestion a de novo biosynthesis by *A. longisetosus*. Unlike other studies (Aboshi et al. 2013; Shimizu et al. 2014; Zhou et al. 2008), our experiments were conducted under axenic conditions and exclude a potential bacterial involvement in 18:2ω6,9-synthesis. From a phylogenetic point of view the de novo synthesis of 18:2ω6,9 in both Astigmata (Aboshi et al. 2013; Shimizu et al. 2014) and Oribatida (this study) is interesting, as this is further evidence for astigmatid mites being nested within oribatid mites (Norton 1994).

Based on our biochemical data (Fig. 2), we propose the following biochemical pathway for C_{18}-fatty acids in *A. longisetosus* and potentially other oribatid mites (Fig. 3): first the labeled glucose is broken down via glycolysis into acetyl-CoA (and other *β-polycarbonyls*), which is subsequently used to synthesize stearic acid (18:0) de novo, which is the substrate to synthesize the oleic acid (18:1ω9). Oleic acid itself might be a precursor to produce 18:2ω6,9 via a Δ-12 desaturase, yet no enzymatic or sequencing data is available to confirm or rejected this hypothesis. Yet, the results of the starvation experiment (Fig. 1; Table 1) — showing a rapid decline of both 18:1ω9 and 18:2ω6,9 — suggest a potential metabolic connection of these fatty acids. To eventually confirm that *A. longisetosus* can produce 18:2ω6,9 from 18:1ω9, genetic resources (genome/transcriptome) are desperately needed to identify candidate enzymes and perform functional studies to reveal the enzymatic toolkit necessary for *A. longisetosus*' fatty acid metabolism. We previously demonstrated a trophic transfer of NLFAs from resources to mites and discussed the potential use of NLFAs as food-web biomarkers (Brückner et al. 2017). This current study on starvation and the biosynthesis of C_{18}-fatty acids refines this view: (i) upon starvation, oribatid mites quickly metabolized (poly)-unsaturated fatty acids, hence the absence of an absolute unsaturated NLFA biomarker (e.g., 16:2ω6,9, 16:3ω3,6,9, or 18:3ω3,6,9; Kühn et al. 2020) might not exclude a certain diet; (ii) as our model mite was able to synthesize both 18:1ω9

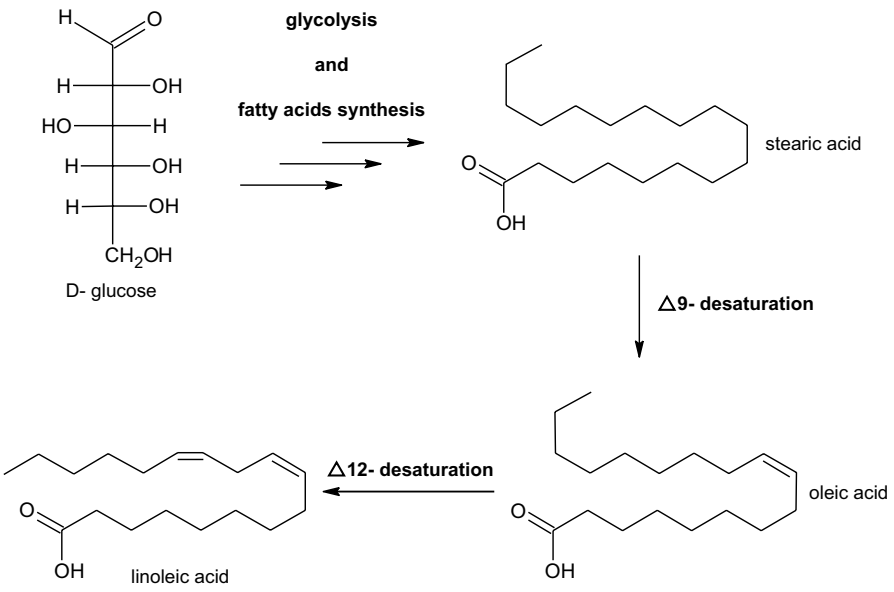

Fig. 3 The proposed biosynthetic pathway leading to C_{18}-fatty acids in the oribatid mite *Archegozetes longisetosus*. Stearic acid (18:0) is initially synthesized from glycolysis products and subsequently double bonds are inserted at positions 9 and 12 yielding oleic acid (18:1ω9) and linoleic acid (18:2ω6,9), respectively

and 18:2ω6,9, the use of these fatty acids as relative biomarkers indicative of a plant and fungal-based diet, receptively (see Kühn et al. 2020) must be carefully evaluated for oribatid mites. Also, as it is hard to control and assess the nutritional state of field-collected oribatid mites, fatty acid patterns retrieved from such animals must be interpreted with extreme caution and further studies on field-caught oribatid mites combining gut-content analysis and fatty acid profiling will be necessary to evaluate the applicability of relative NLFA biomarkers in Oribatida.

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Compliance with ethical standards

Conflict of interest AB and MH declare that they have no conflict of interest.

Ethical approval There are no legal restrictions on working with mites.

References

Aboshi T, Shimizu N, Nakajima Y, Honda Y, Kuwahara Y, Amano H, Mori N (2013) Biosynthesis of linoleic acid in *Tyrophagus* mites (Acarina: Acaridae). Insect Biochem Mol Biol 43:991–996

Behan-Pelletier V (1997) Oribatid mites (Acari: Oribatida) of the Yukon. Insects of the Yukon Biological Survey of Canada (Terrestrial Arthropods). Biological Survey of Canada (Terrestrial Arthropods), Ottawa, pp 115–149

Brückner A, Hilpert A, Heethoff M (2017) Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites. Soil Biol Biochem 115:35–43

Brückner A, Schuster R, Wehner K, Heethoff M (2018) Nutritional quality modulates trait variability. Front Zool 15:50

Bünemann EK et al (2018) Soil quality—a critical review. Soil Biol Biochem 120:105–125

Canavoso L, Bertello L, De Lederkremer R, Rubiolo E (1998) Effect of fasting on the composition of the fat body lipid of *Dipetalogaster maximus*, *Triatoma infestans* and *Panstrongylus megistus* (Hemiptera: Reduviidae). J Comp Physiol B 168:549–554

Canavoso LE, Stariolo R, Rubiolo ER (2003) Flight metabolism in *Panstrongylus megistus* (Hemiptera: Reduviidae): the role of carbohydrates and lipids. Mem Inst Oswaldo Cruz 98:909–914

Canavoso LE, Frede S, Rubiolo ER (2004) Metabolic pathways for dietary lipids in the midgut of hematophagous *Panstrongylus megistus* (Hemiptera: Reduviidae). Insect Biochem Mol Biol 34:845–854

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Hagenfeldt L, Wahren J (1968) Human forearm muscle metabolism during exercise II uptake, release and oxidation of individual FFA and glycerol. Scand J Clin Lab Investig 21:263–276

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:9

Harper GL, King RA, Dodd C, Harwood JD, Glen D, Bruford MW, Symondson WOC (2005) Rapid screening of invertebratepredators for multiple prey DNA targets. Mol Ecol 14:819–827

Haubert D (2006) Unraveling the structure of soil food webs: stable isotope, fatty acid and compound-specific fatty acid analysis. PhD Thesis

Haubert D, Haggblom MM, Scheu S, Ruess L (2004) Effects of fungal food quality and starvation on the fatty acid composition of *Protaphorura fimata* (Collembola). Comp Biochem Physiol B 138:41–52

Haubert D, Häggblom MM, Langel R, Scheu S, Ruess L (2006) Trophic shift of stable isotopes and fatty acids in Collembola on bacterial diets. Soil Biol Biochem 38:2004–2007

Haubert D, Häggblom MM, Scheu S, Ruess L (2008) Effects of temperature and life stage on the fatty acid composition of Collembola. Eur J Soil Biol 44:213–219

Heethoff M, Laumann M, Bergmann P (2007) Adding to the reproductive biology of the parthenogenetic oribatid mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). Turk J Zool 31:151–159
Heidemann K, Scheu S, Ruess L, Maraun M (2011) Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. Soil Biol Biochem 43:229–236
Hilligsøe H, Holmstrup M (2003) Effects of starvation and body mass on drought tolerance in the soil collembolan *Folsomia candida*. J Insect Physiol 49:99–104
Howard R, Stanley-Samuelson D, Nelson D (1993) Insect lipids: chemistry, biochemistry, and biology. University of Nebraska Press, Lincoln
King R, Read D, Traugott M, Symondson WOC (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol Ecol 17:947–963
Kühn J, Tobias K, Jähngen A, Ruess L (2020) Shifting systems: prerequisites for the application of quantitative fatty acid signature analysis in soil food webs. Philos Trans R Soc B 375:20190650
Lavy D, Nedved O, Verhoef H (1997) Effects of starvation on body composition and cold tolerance in the collembolan *Orchesella cincta* and the isopod *Porcellio scaber*. J Insect Physiol 43:973–978
Malcicka M, Visser B, Ellers J (2018) An evolutionary perspective on linoleic acid synthesis in animals. Evol Biol 45:15–26
Maraun M, Erdmann G, Fischer BM, Pollierer MM, Norton RA, Schneider K, Scheu S (2011) Stable isotopes revisited: their use and limits for oribatid mite trophic ecology. Soil Biol Biochem 43:877–882
Maraun M, Augustin D, Pollierer MM, Scheu S (2020) Variation in trophic niches of oribatid mites in temperate forest ecosystems as indicated by neutral lipid fatty acid patterns. Exp Appl Acarol 81:103–115
McCue MD (2012) Comparative physiology of fasting, starvation, and food limitation. Springer, Berlin
Menzel R, Ngosong C, Ruess L (2017) Isotopologue profiling enables insights into dietary routing and metabolism of trophic biomarker fatty acids. Chemoecology 27:101–114
Menzel R, Geweiler D, Sass A, Simsek D, Ruess L (2018) Nematodes as important source for omega-3 long-chain fatty acids in the soil food web and the impact in nutrition for higher trophic levels. Front Ecol Evol 6:96
Norton RA (1994) Evolutionary aspects of oribatid mite life histories and consequences for the origin of the Astigmata. In: Houck MA (ed) Mites: ecological and evolutionary analyses of life-history patterns. Chapman and Hall, New York, pp 99–135
Pollierer MM, Dychmans J, Scheu S, Haubert D (2012) Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Funct Ecol 26:978–990
Pollierer MM, Larsen T, Potapov A, Brückner A, Heethoff M, Dyckmans J, Scheu S (2019) Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecol Monogr 89:e01384
Potapov AM, Tiunov AV, Scheu S (2019) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biol Rev 94:37–59
Price ER, Krokfors A, Guglielmo CG (2008) Selective mobilization of fatty acids from adipose tissue in migratory birds. J Exp Biol 211:29–34
Raclot T (2003) Selective mobilization of fatty acids from adipose tissue triacylglycerols. Prog Lipid Res 42:257–288
Raclot T, Groscolas R (1993) Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism. J Lipid Res 34:1515–1526
Rosumek FB,Brückner A, Blüthgen N, Menzel F, Heethoff M (2017) Patterns and dynamics of neutral lipid fatty acids in ants—implications for ecological studies. Front Zool 14:36
Rosumek FB, Blüthgen N, Brückner A, Menzel F, Gebauer G, Heethoff M (2018) Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids. PeerJ 6:e5467
Ruess L, Chamberlain PM (2010) The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol Biochem 42:1898–1910
Sayanova O, Haslam R, Guschina I, Lloyd D, Christie WW, Harwood JL, Napier JA (2006) A bifunctional Δ12, Δ15-desaturase from *Acanthamoeba castellanii* directs the synthesis of highly unusual n-1 series unsaturated fatty acids. J Biol Chem 281:36533–36541
Schatz H, Behan-Pelletier V, O'Connor BM, Norton RA (2011) Suborder Oribatida van der Hammen, 1968. In: Zhang Z-Q (ed) Animal biodiversity an outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148:7–237
Seastedt T (1984) The role of microarthropods in decomposition and mineralization processes. Annu Rev Entomol 29:25–46

Shimizu N, Naito M, Mori N, Kuwahara Y (2014) *De novo* biosynthesis of linoleic acid and its conversion to the hydrocarbon (*Z*,*Z*)-6,9-heptadecadiene in the astigmatid mite, *Carpoglyphus lactis*: incorporation experiments with ^{13}C-labeled glucose. Insect Biochem Mol Biol 45:51–57

Smith S (1994) The animal fatty acid synthase: one gene, one polypeptide, seven enzymes. FASEB J 8:1248–1259

Stanley-Samuelson DW, Jurenka RA, Cripps C, Blomquist GJ, Derenobales M (1988) Fatty acids in insects—composition, metabolism, and biological significance. Arch Insect Biochem Physiol 9:1–33

Wallis JG, Watts JL, Browse J (2002) Polyunsaturated fatty acid synthesis: what will they think of next? Trends Biochem Sci 27:467–473

Wallwork J (1979) Energetics of soil mites: the experimental approach. In: Proceedings of the 4th international congress on acarology, pp 69--73

Weinert J, Blomquist G, Borgeson C (1993) *De novo* biosynthesis of linoleic acid in two non-insect invertebrates: the land slug and the garden snail. Experientia 49:919–921

Zhou XR, Horne I, Damcevski K, Haritos V, Green A, Singh S (2008) Isolation and functional characterization of two independently-evolved fatty acid Δ12-desaturase genes from insects. Insect Mol Biol 17:667–676