Journal of Functional Foods 68 (2020) 103890

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Montmorency tart cherry (*Prunus cerasus* L.) acts as a calorie restriction mimetic that increases intestinal fat and lifespan in *Caenorhabditis elegans*

David van de Klashorst[a], Amber van den Elzen[a], Jasper Weeteling[a], Michael Roberts[b], Terun Desai[b], Lindsay Bottoms[b], Samantha Hughes[a,*]

[a] HAN BioCentre, HAN University of Applied Sciences, Nijmegen, the Netherlands
[b] Centre for Research in Psychology and Sports Science, University of Hertfordshire, Hertfordshire, UK

ARTICLE INFO

Keywords:
Healthy aging
Pharyngeal pumping
Metabolic Syndrome
Lifespan
Polyphenol
Anthocyanins

ABSTRACT

Montmorency Tart Cherries, MTC, (*Prunus cerasus* L.) possess a high anthocyanin content as well as one of the highest oxygen radical absorbance capacities of fruits at common habitual portion sizes. MTC have been shown to contribute to reducing plasma lipids, plasma glucose and fat mass in rats and strikingly, similar effects are observed in humans. However, there is a paucity of research examining the molecular mechanisms by which such MTC effects are induced. Here, we show that when exposed to MTC, *Caenorhabditis elegans* display an extension of lifespan, with a corresponding increase in fat content and increase in neuromuscular function. Using RNA interference, we have confirmed that MTC is likely to function via the Peroxisome Proliferator-Activated Receptor (PPAR) signalling pathway. Further, consumption of MTC alters the pharyngeal pumping rate of worms which provides encouraging evidence that MTC may be operating as a calorie restriction mimetic via metabolic pathways.

1. Introduction

Modern dietary habits result in a myriad of cardio-metabolic dysfunction leading to the development of Metabolic Syndrome (MetS), and eventually chronic disease. It is estimated that, worldwide, one quarter of the world population, estimated to be over 1 billion people have MetS (Saklayen, 2018), a cluster of cardio-metabolic criteria including obesity, hyperglycaemia, dyslipidaemia and elevated blood pressure (Holubkova, Penesova, Sturdik, Mosovska, & Mikusova, 2012; Saklayen, 2018). MetS is often a precursor to type 2 diabetes and cardiovascular disease, which together place a significant burden on health services and are the leading causes of reduced lifespan and morbidity worldwide (O'Neill, Bohl, Gergersen, Hermansen, & O'Driscoll, 2016). Given the social challenges faced by the prevalence of MetS, obesity, cardiovascular disease and diabetes, non-pharmacological interventions are desperately needed to safely prevent and mitigate the development of these diseases.

Recently, there has been renewed interest into diets supplemented with "functional foods" particularly those rich in polyphenols for health and exercise benefits, namely beetroot juice (Ferreira & Behnke, 2010), purple sweet potatoes (Chang, Hu, Huang, Yeh, & Liu, 2010), blueberries (McAnulty et al., 2011; Wilson et al., 2006), pomegranate juice (Thrombold, Reinfeld, Casler, & Coyle, 2011), pitanga fruit (Tambara et al., 2018), green tea (Jowko et al., 2011) and cherries (Bell, McHugh, Stevenson, & Howatson, 2014; Traustadottir et al., 2009). Ensuring a diet rich in such foods results in significant health benefits to humans, specifically related to their antioxidative, anti-inflammatory, anti-obesity and anti-cancer properties (Ghosh, 2005; Seymour et al., 2009; Wang & Stoner, 2008; Wu et al., 2006). Montmorency Tart Cherries (*Prunus cerasus* L.; MTC), possess a high anthocyanin content and has one of the highest oxygen radical absorbance capacities of fruits consumed at common habitual portion sizes (Ou, Bosak, Brickner, Iezzoni, & Seymour, 2012). The health benefit of MTC is likely due to the presence of polyphenols, mainly anthocyanins, which are commonly found in the skin of the fruit and are responsible for its dark red pigment (Khoo, Azlan, Tang, & Lim, 2017).

Human studies have established that MTC has anti-inflammatory (Bell, McHugh, et al., 2014), anti-oxidative (Bell, McHugh, et al., 2014), anti-hypertensive (Keane, George, et al., 2016) and anti-hyperuricaemic (Bell, Gaze, et al., 2014) properties. Correspondingly, rats fed MTC displayed significantly improved lipid profiles and reduced fat mass, hyperinsulinaemia and hyperglycaemia compared to control animals (Seymour et al., 2008, 2009). Additionally, tart cherries have been shown to have an anti-diabetic effect in diabetic rats, via the reduction

* Corresponding author.
E-mail address: samantha.hughes@han.nl (S. Hughes).

https://doi.org/10.1016/j.jff.2020.103890
Received 19 August 2018; Received in revised form 25 January 2020; Accepted 2 March 2020
Available online 10 March 2020

in plasma glucose (Tahsini & Heydari, 2012). Together, although this is promising evidence that MTC can thwart MetS development, there is a paucity of research examining mechanisms of action by which these effects are induced. Previous studies investigating the mechanisms underpinning MTC used animal models, and showed that MTC induced gene expression of peroxisome proliferator-activated receptors (PPARα/γ) and downregulated IL-6 (interleukin-6) and TNF-α (tumour necrosis factor-alpha) (Seymour et al., 2008, 2009), all major pathways involved in fat metabolism and insulin signalling. It is likely that the phytochemicals in MTC may alter the transcription of other genes involved in the response to oxidative stress (Kirakosyan, Gutierrez, Solano, Seymour, & Bolling, 2018), but these have yet to be fully elucidated. It is therefore obvious that a detailed understanding of the effect of MTC on these, and other, pathways that lead to positive health effects is lacking.

We have used the model organism *Caenorhabditis elegans* to study the positive health benefits provided by MTC. *C. elegans* is a powerful model with which to study the molecular pathways that underpin human disease (Markaki & Tavernarakis, 2010; Shaye & Greenwald, 2011b) but also with which to investigate functional foods. *C. elegans* is a small, transparent nematode worm with many well-studied molecular networks and tissue systems that are also found in vertebrates (Kaletta & Hengartner, 2006; Lehner, Crombie, Tischler, Fortunato, & Fraser, 1996; Shaye & Greenwald, 2011a; Sulston & Horvitz, 1977). The nematode has been fully sequenced and mapped (Hiller et al., 2005), with 80% of human genes possessing homologs in *C. elegans* (Kaletta & Hengartner, 2006). In addition, feeding behaviour, nutritional uptake and fat metabolism are conserved between *C. elegans* and humans (Hashmi et al., 2013). Importantly, *C. elegans* is frequently used to investigate the biological functions of food compounds (Buchter et al., 2013; Chen, Muller, Richling, & Wink, 2013; Gao et al., 2015; Grunz et al., 2012; Kock, Weldle, Baler, Buchter, & Watjen, 2019; Tambara et al., 2018; Wilson et al., 2006). Taken together, these factors emphasise the translational relevance of this model species and its potential to predict effects in higher animals and inform clinical nutrition practice in humans.

We have chosen to use *C. elegans* to better understand the molecular basis of the health promoting effects of MTC by examining the response of *C. elegans* to various dilutions of MTC concentrate. We hypothesised that exposure to MTC will increase lifespan and reduce fat content. We also used RNAi to silence genes involved in fat metabolism, conserved between humans and nematodes, to provide insight into the molecular pathways by which MTC acts. Lastly, using pharyngeal pumping assays, we provide evidence that MTC does act as a calorie restriction mimetic.

2. Materials and methods

2.1. Strains and maintenance of worms

Strains used in this study (wild type *N2* var. Bristol and *TJ356* [*zIs356* IV*(pdaf-16::daf-16::gfp; rol-6)*]) and bacterial strains were provided by the *Caenorhabditis* Genetics Centre (CGC). *C. elegans* strains were maintained on Nematode Growth Media (NGM) agar prepared according to standard protocols (Brenner, 1974) and plates seeded with *OP50 E. coli* as a bacterial food source.

To synchronise worm populations, gravid worms were washed from plates with M9 buffer (Brenner, 1974) and bleached according to standard protocols (Stiernagle, 2006) using alkaline hypochlorite solution (4 ml 5% sodium hypochlorite, 1 ml 4 M sodium hydroxide, 5 ml dH_2O). Released eggs were left to hatch overnight at room temperature (19–20 °C) in M9 buffer in the absence of a food source, giving rise to a population of synchronised L1 larvae which could then be placed directly onto NGM to develop at a similar rate.

2.2. MTC concentrate seeded NGM

Concentrated Montmorency Tart Cherry juice was obtained from Cherry Active (now called Active Edge, 5–060142-250010, Hanwell, UK). This product is 100% concentrated Montmorency Cherry juice with no added preservatives. Cherry Active suggests that the recommended daily human consumption is 30 ml MTC concentrate mixed with 240 ml water. Total phenolic content of Cherry Active MTC was determined to be 726 mg/ml by the Folin-Ciocalteu assay (Swain & Hillis, 1959) using a gallic acid standard curve.

Concentrated MTC Juice (Cherry Active) was added directly to the cooled molten NGM prior to pouring plates. The final concentrations of MTC juice in the NGM plates ranged from 16.7 µl/ml to 150 µl/ml in NGM. The pH of all preparations was checked, substituting water for NGM. For each MTC spiked water sample, there was no change in pH compared to the control.

2.3. Development assay

Two assays were undertaken, both adapted from Xiong *et al.* (Xiong, Pears, & Woollard, 2017). The first was a simple lawn clearance based where single L4 animals were placed on 2 ml NGM, with or without MTC, in a 12-well plate containing 30 µl *OP50*. This was classed as day 0. The plates were monitored daily for clearance of the bacterial lawn. In the second assay, L1 animals were added to plates, prepared in the same way as previously, and each day the worms were observed for development. The addition of L1 animals to the plates was at time 0. Worms were scored as younger than L4 (< L4), L4 or egg laying. Worms that were dead were also scored.

2.4. RNA interference

RNA interference experiments were performed using standard protocols (Kamath & Ahringer, 2003). NGM for RNAi was supplemented with 116.7 µl/ml of MTC concentrate in NGM, or an equivalent volume of distilled water as the control. The RNAi clones were chosen based on their homology to human genes involved in different aspects of fat metabolism (Ashrafi et al., 2003; Shaye & Greenwald, 2011b). Clones were obtained from the Vidal ORFeome-based RNAi library (Rual et al., 2004) and those used in this research are listed in Table 1. All nematode experiments were conducted using the *N2* strain. dsRNA was delivered by feeding to age-synchronised L1 stage animals, which were incubated at 20 °C and phenotypes were observed 48 h later, when control animals had reached the L4 stage. Control RNAi was performed using *HT115* bacteria transformed with an empty *L4440* vector.

2.5. Lifespan assay

To assess lifespan, we used wild type *N2*. Animals were reared on *OP50* seeded NGM at 16 °C following standard protocols. When gravid, animals were bleached and the resulting age synchronised L1 animals were placed onto seeded NGM. When animals were L4, this was counted as Day 0 of the survival assay. During the reproductive period, the worms were transferred to a new plate and observed every day for death. Animals were scored as dead if they failed to respond to a touch by a platinum wire. Survival curves were generated and data analysed using the OASIS software (Yang et al., 2011). For multiple analysis, $p < 0.0055$ was considered as significant (Bonferroni corrections).

2.6. Obesity assay

The obesity assay was adapted from Mak, Nelson, Basson, Johnson, and Ruvkun (2006) and Ashrafi et al. (2003). A stock solution of Nile Red (Roth) was prepared at 0.5 mg/ml in acetone and diluted in 1xPBS to 1 µg/ml, both solutions were stored in the dark at 4 °C. Nile Red was added to molten NGM (1 ml of working solution in 50 ml NGM) and

Table 1
The effect of silencing genes associated with fat metabolism in the presence of Montmorency tart cherry juice. Wild type animals were placed onto RNAi NGM seeded with, or without, 116.7 μl/ml MTC, and after 48 h at 20 °C the developmental progress of the animals was compared. Control animals that are not exposed to MTC and are fed control RNAi (empty vector *L4440*) reach the L4/young adult stage (as shown by a green highlighted box and "normal development to L4/YA"). In contrast, MTC exposed worms have a significant developmental delay as indicated by a red box stating "enhanced developmental delay". A selection of genes concerning fat metabolism are silenced in the presence of MTC and the development of the worms compared to the control was investigated. The silencing of most genes in the presence of MTC have no effect on developmental rate, while others show a recovery of the developmental delay (highlighted in green) and some further enhance the developmental delay (highlighted in red).

Worm gene name	Development after 48hours RNAi	Human gene/description
L4440	Normal development to L4/YA	Empty vector control
L4440 + MTC	Developmental delay	Empty vector control + MTC
acl-12	Developmental delay	Ortholog of LPGAT1; Acyl-CoA:lysophosphatidylglycerol acyltransferase 1
alh-11	Developmental delay	4-trimethylaminobutyraldehyde dehydrogenase
C17C3.3	Enhanced developmental delay	Acyl-coenzyme A thioesterase 8
C37H5.13	Developmental delay	Acyl-coenzyme A thioesterase 8
cyp-13A11	Developmental delay	A nematode cytochrome P450
daf-22	Normal development to L4/YA	Isoform SCPx of Non-specific lipid-transfer protein
daf-7	Developmental delay	Gene involved in fat metabolism
eat-2	Enhanced developmental delay	Neuronal acetylcholine receptor subunit alpha-7
elo-4	Normal development to L4/YA	Polyunsaturated fatty acid elongase for very long chain fatty acids
F09C8.1	Enhanced developmental delay	Phospholipase B1, isoform 5, membrane-associated
F25E2.3	Developmental delay	Acyl-coenzyme A thioesterase 8
fard-1	Developmental delay	Fatty acyl-CoA reductase 1
fat-3	Enhanced developmental delay	fatty acid desaturase 1
fat-4	Developmental delay	fatty acid desaturase 1
H27A22.1	Normal development to L4/YA	Glutaminyl-peptide cyclotransferase-like protein
klf-1	Enhanced developmental delay	highly similar to Krueppel-like factor 5
lipl-1	Enhanced developmental delay	gastric triacylglycerol lipase isoform 1
lipl-4	Normal development to L4/YA	Lipase member M precursor
M79.2	Developmental delay	Glycerol-3-phosphate acyltransferase 3
nhr-49	Enhanced developmental delay	Isoform 1 of Hepatocyte nuclear factor 4-gamma
nhr-80	Developmental delay	Isoform HNF4-Alpha-2 of Hepatocyte nuclear factor 4-alpha
oga-1	Developmental delay	O-linked N-acetylglucosamine (O-GlcNAc)-selective N-acetyl-beta-D-glucosaminidase (O-GlcNAcase)
sbp-1	Normal development to L4/YA	SREBP
T19D7.7	Developmental delay	Phospholipase B1, isoform 5, membrane-associated
tub-1	Normal development to L4/YA	Isoform 1 of Tubby protein homolog

when solid the plates were seeded with *E. coli OP50*. Age synchronised L1 worms were added to the plates and allowed to develop to L4, at which point the worms (n = 26 per condition) were sacrificed for microscopy.

Individual worms were mounted onto 2% agarose pads in 0.1% sodium azide. Fluorescent imaging was carried out using a Zeiss Imager.M2 microscope and photomicrographs were taken using a x63 objective (Zeiss) and Zeiss Zen 2012 Blue Software. Images were taken using the same gain and exposure settings of animals on the same focal plane (at the grinder in the second pharyngeal bulb). Using ImageJ software, the fluorescence in a 20x20 pixel box placed at the grinder was calculated, to give a value corresponding to level of staining of the Nile Red vital dye. The arbitrary values for "red" are equivalent to the level of Nile Red staining, which are normalised to the control worms and plotted with standard deviation error bars. The data is analysed using the *t*-test.

2.7. Microscopy

DIC images were taken using a Leica benchtop M165FC microscope equipped with a Leica DFC425C camera and photomicrographs taken using LAS V4.2 software. To take fluorescent images, worms were mounted onto 2% agarose pads in 0.1% sodium azide. Fluorescent imaging was carried out using a Zeiss Imager.M2 microscope and images taken using Zeiss Zen 2012 Blue Software. All images were compiled using Adobe Photoshop 7.0 and backgrounds merged.

2.8. Pharyngeal pumping

Gravid animals were subjected to sodium hypochloride treatment, and synchronised L1 nematodes placed directly onto NGM in the presence or absence of MTC (at either 16.7 µl/ml or 66.7 µl/ml). When animals had reached L4 stage, worms were washed off the plates with M9 buffer. After removal of the bacteria, worms were resuspended in M9 and serotonin (10 mM final concentration) to stimulate pumping. After 10 min incubation in serotonin, electropharyngeogram recordings were taken using the NemaMetrix ScreenChip system using an SC30 chip. Each recording was 2 min in duration and analysed using the NemaMetrix ScreenChip software in 2 independent experiments. The data was combined for each MTC condition and the averages and standard error of the mean plotted for pump frequency, duration, inter pump interval and amplitude with a *t*-test used for statistical analysis.

2.9. Localisation of DAF-16::GFP

Intracellular localisation of DAF-16 was observed using the strain *TJ356*. Synchronised L1 animals were placed on MTC spiked NGM and allowed to develop to L4. At this point, 100 animals were assessed for DAF-16::GFP localisation using a Leica M165FC microscope, based on the method described in Buchter et al. (2013).

2.10. Pluronic gel mobility assay

Pluronic F-127 is a biocompatible thermoreversable hydrogel which is a favourable environment in which to perform a burrowing assay (Lesanpezeshki et al., 2019). Worms are age synchronized and grown to L4 stage, at which point they are transferred to NGM spiked with MTC (to 16.7 µl/ml and 66.7 µl/ml) and FuDR at a final concentration of 10 mM. The plates are incubated at 20 °C for 1 week at which point the worms are used for the pluronic gel assay.

A gel concentration of 26% (*w/w* with distilled water) is prepared and a droplet added to the bottom of a 12-well plate. Worms (wild type *N2*) are added to the droplet, and then pluronic gel added up to a height of 700 mm. To the surface of the well, *OP50* and 1% isoamyl alcohol (25 µl) is added, when time is recorded as time 0. The wells are monitored every 15 min over a 2 h period, and the number of worms at the surface of the well counted. Each experiment is performed in triplicate with 30 worms in 2 independent experiments.

3. Results and discussion

3.1. Relating human dose to C. elegans

Various human studies have investigated the effect of MTC on humans, however there is very little coherence in MTC dosing between studies. The lowest dilution used in a trial is 30 ml MTC with 220 ml water, equivalent to 120 µl MTC/ml water, henceforth described as 120 µl/ml (Lynn et al., 2014), while other studies use 30 ml MTC in 100 ml water (230 µl/ml) (Desai, Bottoms, & Roberts, 2018; Desai, Roberts, & Bottoms, 2019) up to a maximal dilution of 60 ml MTC in 100 ml water (375 µl/ml) (Keane, George, et al., 2016; Keane, Haskell-Ramsey, Veasey, & Howatson, 2016). To further compound the issue, the recommended daily dose as prescribed by Cherry Active is mixing 30 ml MTC with 240 ml water (equivalent to 111 µl/ml). Moreover, despite pharmacokinetic studies with MTC examining anthocyanin and phenolic concentrations in systemic circulation (Bell, Gaze, et al., 2014; Keane, Bell, et al., 2016; Seymour et al., 2014), there is no evidence of how much MTC is being absorbed into tissues in humans, although this has been investigated in healthy rats (Kirakosyan et al., 2015).

Therefore, to try to equate these human consumption doses to *C. elegans*, a dose response curve was undertaken using development as an end point. MTC concentrations ranged from 16 µl/ml to 300 µl/ml, however, worms did not survive on MTC concentrations above 150 µl/ml (*data not shown*) and so these were not tested further. Nematodes exposed to low dilutions of MTC developed at a similar rate to controls, until the MTC concentration was 66 µl/ml or more (Fig. 1). At 66.7 µl/ml MTC, the nematodes displayed a delayed development compared to control animals, but did reach the egg laying stage. In contrast, few animals were able to reach egg laying age when exposed to 100 µl/ml MTC or more (Fig. 1). For this reason, we chose to focus our efforts on those MTC dilutions that were relevant for *C. elegans* (i.e. up to 100 µl/ml). In this way, it is possible to comment on the general health promoting benefits of MTC. However, this does highlight a problem when linking the data observed in *C. elegans*, and other model organisms. However, investigating the general health promoting effects of MTC is valuable using *C. elegans* as it provides an indication of the possible mechanistic basis for the health promoting effects (Desai et al., 2018, 2019; Seymour et al., 2014).

3.2. Lifespan is increased following exposure to MTC

Studies have shown that longevity is related to a metabolic shift away from glucose metabolism towards lipid oxidation (van Heemst, 2010). Therefore, we were interested to see if exposure to MTC has any effect on lifespan. Wild type animals that were not exposed to MTC lived for a maximum of 17 days post L4, while animals exposed to MTC displayed a small but significant increase in lifespan (Fig. 1, Table 2). This extension to lifespan was most striking at lower MTC dilutions, 16.7 µl/ml of MTC, the longest-lived animal was 23 days old and at 150 µl/ml, the maximum lifespan was 20 days (Fig. 1, Table 2). Intriguingly, the mean lifespan was only extended for nematodes exposed to the lower doses of MTC (16.7 µl/ml), with higher levels of MTC having a mean lifespan less than that of control animals.

Together, our data shows that, at least concerning lifespan, lower dilutions of MTC are more beneficial to the worms than higher dilutions. It is possible that a hormetic effect of MTC is occurring, where the benefit provided by lower levels of anthocyanins, the main polyphenols in MTC, is to augment endogenous antioxidant capacity and scavenge free radicals more effectively, while at higher concentrations the pro-oxidant nature of the anthocyanins reverses some health-promoting effects (Blando, Gerardi, & Nicoletti, 2004; Konczak & Zhang, 2004).

Such a lifespan extension as observed here is similar to that of blueberry polyphenols, which were able to increase lifespan and delay aging in *C. elegans* (Wilson et al., 2006). This may be related to the fact that despite tart cherries containing sugar, which would be expected to reduce lifespan, the fruit is able to reduce glycaemic stress by modulating the insulin signalling pathway (Seymour et al., 2009). Such a reduction in glycaemic stress may be mediated by the presence of MTC anthocyanins, phenolic acids and/or their secondary metabolites (Kirakosyan, Seymour, Urcuyo-Lianes, Kaufman, & Bolling, 2009; Scazzocchio et al., 2011), which may reduce the concentration of ligands available to activate the insulin signalling cascade (see Fig. 2).

3.3. Lifespan extension by MTC exposure is not mediated by DAF-16/FoxO

To assess if the lifespan extension provided by MTC is a result of DAF-16 signalling, we observed the localisation of DAF-16. Our results show that MTC did not have a striking effect on DAF-16::GFP localisation at all MTC concentrations tested, with localisation being predominantly cytoplasmic (Fig. 3). This suggests that the MTC is not activating stress response genes and it is also unlikely that the lifespan extending effects are due to activation of the insulin signalling pathway.

3.4. MTC acts though the PPAR signalling pathway

As there is significant homology between the mechanisms of fat storage and regulation in *C. elegans* and mammals (Ashrafi et al., 2003; Hashmi et al., 2013), it is possible to unravel the pathways through which MTC acts. We chose a number of candidate genes based on their

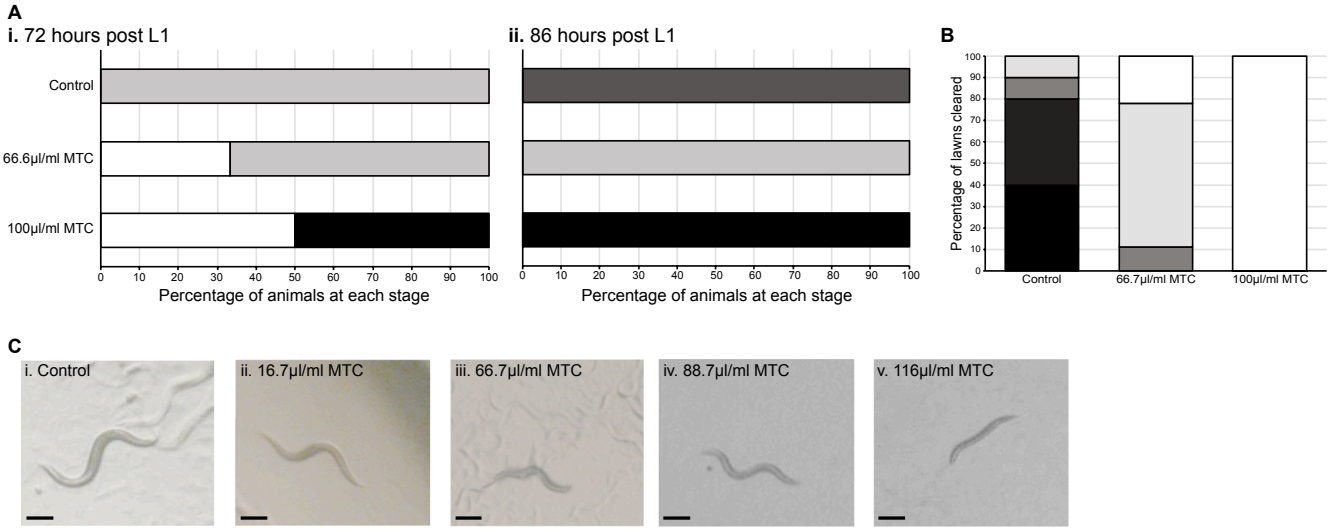

Fig. 1. MTC exposure causes a developmental delay at elevated concentrations. (A) Synchronised worms were placed on NGM (time = 0 h) and observed daily. Worms were assayed for their development at 72 (i) and 86 (ii) hours post L1. Worms were assessed as being at a stage younger than L4 (white bars), L4 (light grey bars) or egg laying adults (dark grey bars). Worms which died were recorded (black bars). Worms that were exposed to MTC concentrations above 100 µl/ml died within 90 h post development and are not shown. (B) A lawn clearance assay was undertaken as a proxy for brood size. L4 animals were added to NGM (time = day 0) and observed daily . The day on which the lawn was cleared was recorded. Clearance on day 4 are the black bars, clearance after 5 days are very dark grey bars, while grey bars indicate a cleared lawn after 7 days. Light grey bars show where the lawn is cleared after 8 days. Where the bars are white, shows where it took 9 days or more to clear the lawn. No lawns were cleared on day 6. (C) Representative images of worms at different MTC concentrations after 72 h growth on MTC from L1 stage. (i) Control animals are gravid. (ii) Animals exposed to 16.7 µl/ml MTC have a similar development to control worms. (iii) After 66.7 µl/ml and (iv) 88.7 µl/ml MTC exposure, animals have a small developmental delay. (v) Worms exposed to 116.7 µl/ml have a significant developmental delay. This animal is not dead, but has not yet reached the L4 stage and is clearly smaller than the other animals. Scale bar, 200 µm.

Table 2
Statistical analysis of lifespan. Raw lifespan data was analysed using the OASIS software (Yang et al., 2011) which provides the mean lifespan with standard error and 95% confidence interval (C.I.). For lifespan plots, see Fig. 2. The control animals (no MTC exposure), lived for a maximum of 17 days, which was extended in all MTC exposed worms. Of the animals exposed to 16.7 µl/ml MTC, 50% mortality was extended from 10 to 13 days, and was the only dilution of MTC to show this. Intriguingly, the mean lifespan was only increased in animals exposed to the lowest dose of MTC, 16.7 µl/ml. For each condition, over 100 animals were used, bar 150 µl/ml where 80 animals were used. The Bonferroni correction was applied to show where the changes to lifespan were most significant.

MTC concentration	Number of worms	Age in days at % mortality		Restricted mean			Bonferroni *p* value
		50%	100%	Days	Std. Error	95% C.I.	
0 µl/ml	112	10	17	11	0.31	10.07–11.27	–
16.7 µl/ml	111	13	23	12	0.43	11.44–13.12	0.0001
66.7 µl/ml	137	9	20	10	0.32	8.96–10.22	0.2358
150 µl/ml	81	8	20	9	0.35	7.90–9.29	0.0001

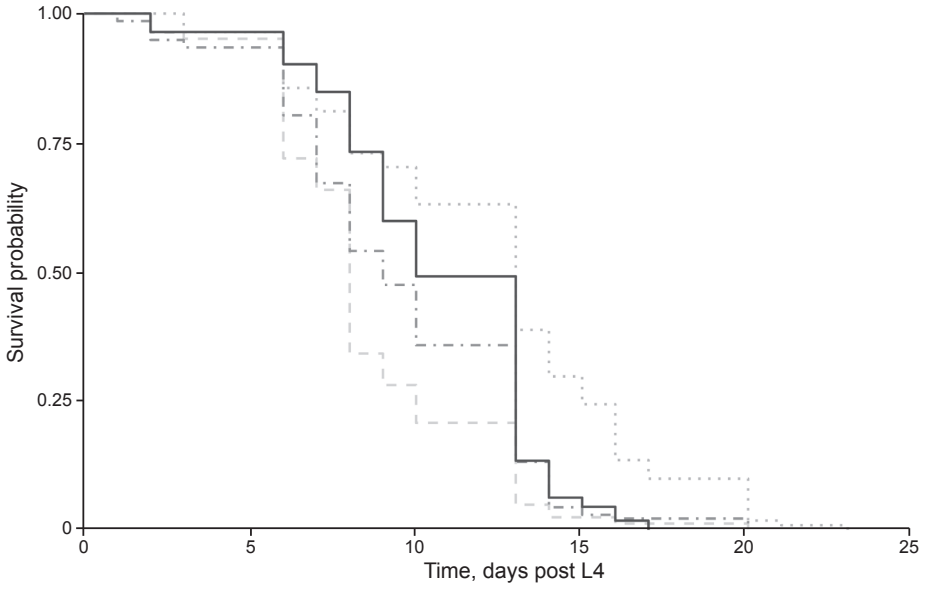

Fig. 2. Lifespan of worms was extended following exposure to MTC. Wild type worms lived for a maximum of 17 days post L4 (solid black lines). Animals exposed to 16.7 µl/ml displayed an extension to lifespan throughout (grey dotted line). In contrast, animals exposed to 66.7 µl/ml (dashed grey line) and 150 µl/ml (solid grey line) showed a faster death rate compared to the control animals, but did have a slight extension to maximum lifespan. For each condition, over 100 animals were used, with the exception of 150 µl/ml MTC where 80 animals were used. For statistical analysis, see Table 2.

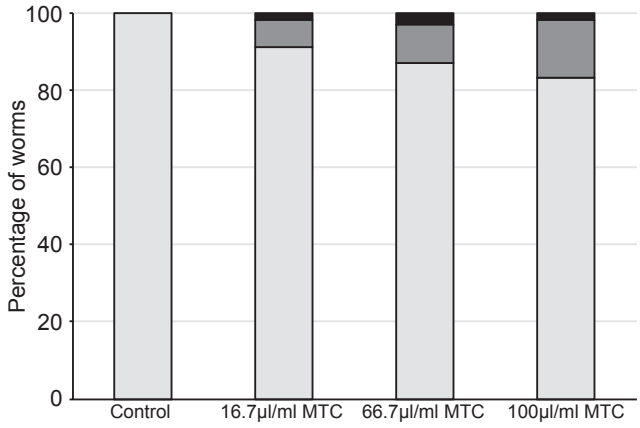

Fig. 3. DAF-16 localisation is not affected by MTC. DAF-16 translocates from the cytoplasm to the nucleus to activate genes concerned with stress resistance and insulin signalling. Localisation of DAF-16::GFP (strain, *TJ356*) was scored as cytosolic (light grey), intermediate (dark grey) or nuclear (black). Under control conditions, DAF-16::GFP is cytoplasmic however, there is a small shift in localisation when exposed to MTC. MTC exposure results in 7–15% animals having intermediate DAF-16::GFP and 3% nuclear localisation. $n = 100$ worms.

homology to key human fat metabolism processes to silence by RNAi (Table 1). During early experiments, a significant developmental delay at L4 was noted at MTC concentrations above 100 µl/ml. Thus, the genes of interest were silenced and after 3 days at 20 °C, the developmental stage of animals were compared to control worms which had reached L4.

The majority of the genes tested showed a similar effect to the control RNAi, *L4440*, in the presence of MTC, in that all animals reached the L4 stage at approximately the same time. However, 7 genes that were silenced in the presence of MTC showed a similar stage of development as the control in the absence of MTC i.e. a recovery of the developmental delay and a further 7 resulted in an enhancement of the developmental delay caused by MTC alone (Table 1).

RNAi knockdown of *daf-22* in the presence of MTC reversed the developmental delay that is caused by exposure to MTC, while on the other hand knockdown of *nhr-49* further enhanced the developmental delay compared to the control. Such an effect is intriguing, as both *daf-22* (nematode homolog of human SCP2) and *nhr-49* (the HNF4G/PPARα homolog) function in the PPAR (peroxisome proliferator-activated receptor) signalling pathway (Dansen et al., 2004; van Gilst, Hadjivassiliou, Jolly, & Yamamoto, 2005), which are central regulators of fat metabolism (Nunn, Bell, & Barter, 2007). Therefore, we are able to confirm that MTC influences the PPAR pathway, as shown previously (Seymour et al., 2008) and thus is likely to operate as a calorie restriction mimetic (Corton et al., 2004).

It is striking that when *sbp-1* (the nematode homolog of SREBP1c, Sterol regulatory element binding protein) is silenced in the presence of elevated MTC dilutions there is a recovery of the developmental delay. In contrast, there is an enhancement of the MTC induced developmental delay when *lipl-1* (a lysosomal lipase) is knocked down. Both *sbp-1* and *lipl-1* have recently been shown to be regulated by the MAX-3 transcription factor, to modulate lipid metabolism in nematodes (Mejia-Martinez et al., 2017). It is likely that these genes form part of a complex network modulating the response to carbohydrates (including glucose) and fat, as suggested previously.

Ultimately, regulation of longevity and lipid metabolism is complex, involving multiple genes and signalling pathways and how longevity and fat accumulation are uncoupled from diet remains elusive. To elucidate the complete mechanism behind the positive health benefits

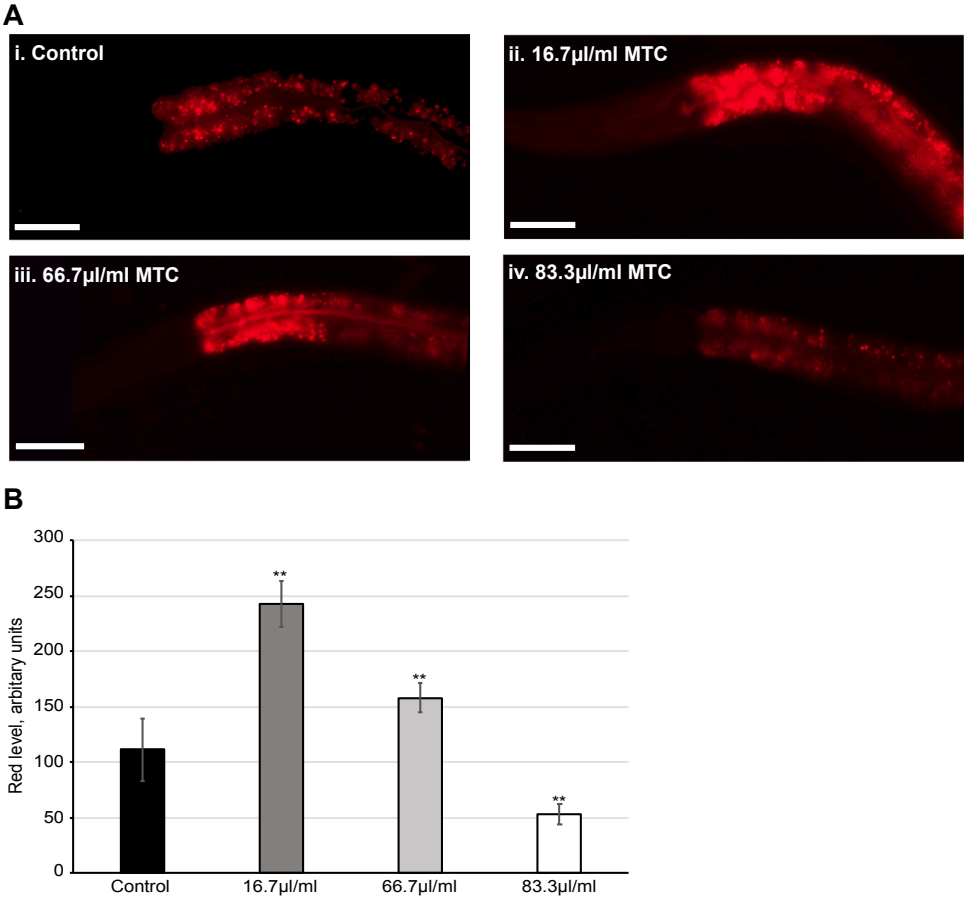

Fig. 4. MTC exposed *C. elegans* show an increase in fat content. (A) Representative images of worms exposed to MTC. In all cases, animals were grown from L1 to L4 on NGM supplemented with increasing dilutions of MTC and the vital dye Nile Red. At L4 stage, worms were mounted for microscopy and imaged using identical settings. Images were taken at the anterior of the animal, with the focal plane always on the valve of the grinder. (i) Control animals, (ii) 16.7 µl/ml MTC, (iii) 66.7 µl/ml MTC and (iv) 83.3 µl/ml MTC. Anterior is to the left, ventral to the bottom. Scale bar is 500 µm. (B) Graph to show the relative fat staining in worms exposed to MTC. Images of worms exposed to MTC were analysed using ImageJ and the values of Nile Red staining are normalized to the control, and this value plotted. Control animals (black bars) show some Nile Red staining of the upper intestine. There is a significant increase in fat staining in the animals exposed to 16.7 µl/ml and 66.7 µl/ml MTC (grey bars) compared to control worms (black bar), while those worms exposed to 83.3 µl/ml MTC (white bar) have a significant reduction in fat staining. Bars are averages and error is standard deviation. Statistical analysis utilized the *t*-test where ** is $p < 0.01$ and $n = 26$.

of MTC is beyond the scope of this work, but will provide a wealth of information linking various aspects of diet, lifespan and health which will have significant impact on the dietary advice given to humans suffering with MetS.

3.5. Exposure to high MTC dilutions enhances lipid staining

Fat metabolism and lifespan are intertwined and likely to be directly coupled (Hansen, Flatt, & Aguilaniu, 2013). To explore the effect of MTC on fat accumulation, we therefore measured the fat content of MTC exposed animals using the vital dye, Nile Red (Ashrafi et al., 2003; Mak et al., 2006). Strikingly, animals exposed to the lower doses of MTC (16.7 µl/ml and 66.7 µl/ml) displayed a significantly increased level of Nile Red staining compared to controls (Fig. 4). In contrast, elevated levels of MTC significantly reduced Nile Red staining (83.3 µl/ml).

Similar to our results, other long-lived mutants display an increase in fat accumulation (O'Rourke, Soukas, Carr, & Ruvkin, 2009). Although MTC has an elevated carbohydrate content, this would prompt the activation of transcription factors that promote the expression of antioxidant or detoxification enzymes (Pang, Lynn, Lo, Paek, & Curran, 2014), thus allowing animals to promote longevity, as shown previously in blueberries (Wilson et al., 2006), supporting our hypothesis for the hormetic action of MTC anthocyanins. As lipids are more energy-dense than carbohydrates, the storage of lipids provides a survival advantage under calorie restriction conditions (van Heemst, 2010).

3.6. Pumping

Moderate dietary restriction in *C. elegans* is usually achieved by reducing food intake by altering the pharyngeal pumping rates (Avery, 1993) and such dietary restriction is able to extend lifespan (Greer & Brunet, 2009; Lakowski & Hekimi, 1998). We measured the pharyngeal pumping rate of L4 stage *C. elegans* grown in the presence or absence of MTC (at 16.7 µl/ml and 66.7 µl/ml). We found that the pumping frequency was reduced following exposure to MTC (Fig. 5). Strikingly, the pump duration, interpump interval and amplitude was increased in a dose dependent manner when worms were exposed to MTC (Fig. 5). Together this suggests that MTC has a calorie restriction effect on *C. elegans*.

3.7. Mobility is enhanced by MTC

One interesting aspect of MTC research, is that it is thought to aid in muscle recovery after exercise (Bell, Stevenson, Davison, & Howatson, 2016; Howatson et al., 2010; Levers et al., 2015, 2016). Worms have only recently been shown to be suitable to use to investigate exercise and how this can be matched to humans (Laranjeiro, Harinath, Burke, Braeckman, & Driscoll, 2017). For this reason, we chose to test the ability of MTC to affect the burrowing capacity of *C. elegans* (Lesanpezeshki et al., 2019). Worms that are 1-week old have a striking reduction in their capacity to burrow through agar to reach a chemoattractant compared to L4 animals (Fig. 6). However, exposure to just 16.7 µl/ml of MTC from L4 for 1 week significantly enhanced the ability of animals to reach the surface of the gel (Fig. 6). This was lost in

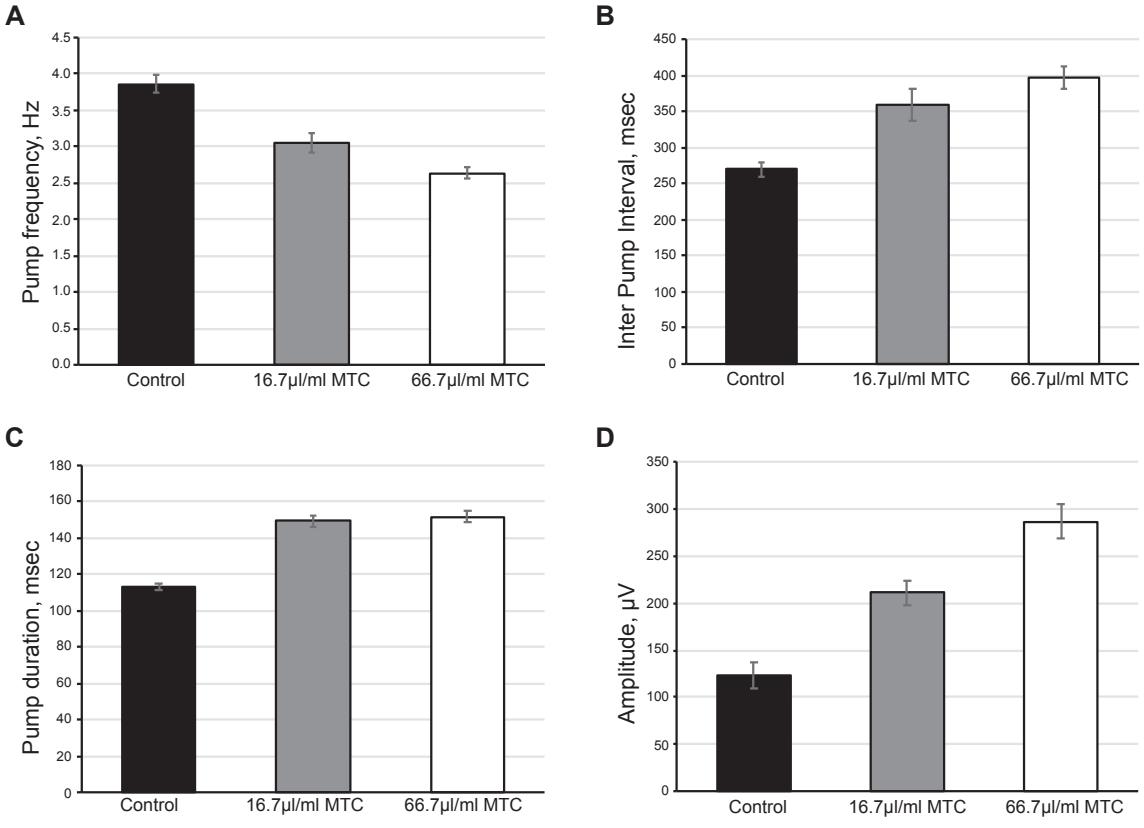

Fig. 5. Analysis of pharyngeal pumping activity when animals are grown in the presence of MTC. In all cases, L4 animals were screened in 2 independent experiments. Wild type animals are used in all cases. Control conditions are shown in black bars ($n = 35$), 16.6 µl/ml MTC are shown in grey ($n = 44$) and 66.7 µl/ml MTC are shown in white bars ($n = 43$). Data is recorded as the average ± S.E.M, with all values being significantly different ($p < 0.001$) unless otherwise stated. (A) Pump frequency, in Hz, is reduced in a dose dependent manner in worms that have been exposed to MTC. (B) Interpump interval, msec, issignificantly increased in worms that are exposed to MTC concentrations. There is no difference in IPI in worms exposed to MTC. (C) Pump duration, in msec, is significantly increased in MTC exposed worms compared to control conditions. There is no difference in pump duration between MTC exposed worms. (D) Amplitude in µV is significantly increased in animals exposed to MTC, in a dose dependent manner.

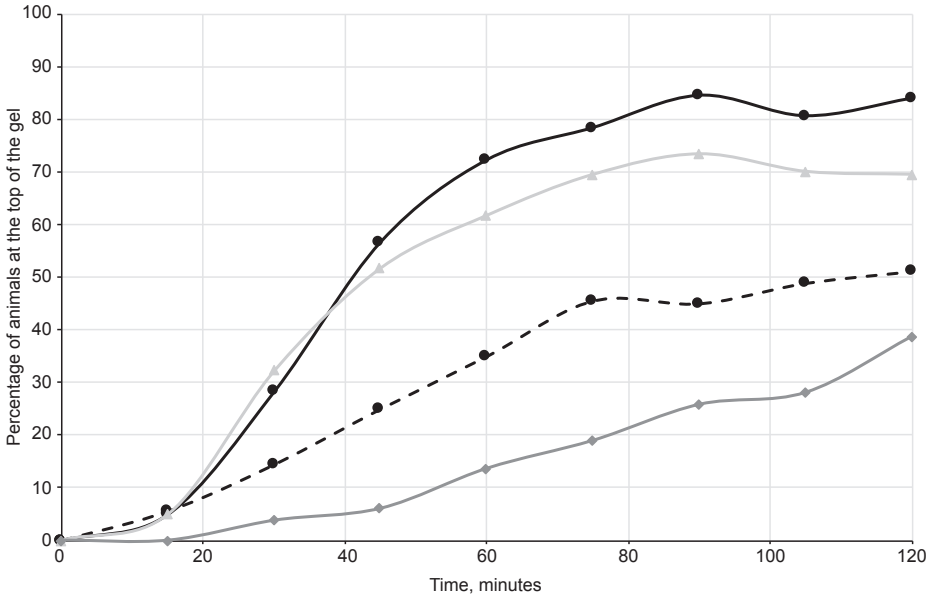

Fig. 6. MTC enhances neuromuscular behaviour at low doses. Animals will move from the bottom of the gel to the top, where there is a chemoattractant, and food. The majority of young, L4, animals (black line, circles) will reach the top of the gel within 90 min. However, older animals have muscle degeneration which can be viewed by fewer worms reaching the surface of the gel. Only half of the 1-week post L4 adults (dashed black line, circles) reach the surface of the gel after 2 h. Interestingly, animals which have been exposed to 16.7 μl/ml MTC (light grey line, triangles) for 1 week have a similar rate of burrowing to the surface as control worms. In contrast, 1-week old animals from 66.7 μl/ml MTC (dark grey line, diamonds) are worse than the similarly ages control worms, with just 40% animals reaching the surface of the gel. n = 180 over 2 independent experiments.

animals exposed to higher doses of MTC. The pluronic gel burrowing assay could be considered an endurance challenge to the worms, who are more accustomed to moving on the surface of a plate. Strikingly, MTC has been shown in humans to aid in performance during endurance challenges (Levers et al., 2015, 2016), which is in sound conclusion with our data.

4. Conclusion

To date, there is promising evidence that MTC can contribute to the prevention of MetS development (Reis et al., 2016), although there is a lack of research examining the mechanism of action by which such effects are induced. Using *C. elegans*, our data provides encouraging evidence that MTC may be operating as a calorie restriction mimetic. Specifically, MTC is likely to act via the PPAR metabolic pathway, as has been shown previously in rats (Seymour et al., 2008) and is in sound agreement with other anthocyanins (Scazzocchio et al., 2011). Indeed, it is highly likely that the mechanism of action of PPAR activation is to operate as a calorie restriction mimetic (Corton et al., 2004) further supporting our findings.

Additionally, our data supports the hormetic action of MTC. We have shown that worms exposed to MTC have an increase in fat content, and since lipids are highly susceptible to oxidative damage, the ability of worms to store more lipid indicates an increased resistance to oxidative stress. In recent years, lipid storage has been shown to be tightly coupled to suppression of oxidative stress and inflammation (van Heemst, 2010). Reduced oxidative stress and inflammation after consuming MTC was shown in older human adults (Chai, Davis, Zhang, Zha, & Firschner, 2019; Johnson et al., 2017; Traustadottir et al., 2009), providing translational evidence to the responses observed here in *C. elegans*.

Together, this data highlights the use of *C. elegans* to search for nutritional interventions of health. Therefore, the result of this work is to provide a basis for further mammalian studies. Indeed, such data can then be used to design and perform more targeted longitudinal clinical trials in MetS humans. In addition, it is possible that genes identified in *C. elegans* as having a role in obesity (or diabetes) are likely to be implicated in human disease as well or, at the very least, provide candidates for anti-obesity and anti-diabetic drugs.

Ethical statement

This research did not use any human subjects. This research did not include any experiments on vertebrate animals. For experiments on *Caenorhabditis elegans*, no permission of an animal ethics committees is necessary, however all documentation is in place at the HAN University of Applied Sciences for handling of *C. elegans*.

CRediT authorship contribution statement

David van de Klashorst: Investigation, Methodology, Validation, Visualization. **Amber van den Elzen:** Investigation, Visualization. **Jasper Weeteling:** Investigation, Visualization. **Michael Roberts:** Conceptualization, Formal analysis, Funding acquisition, Writing - review & editing. **Terun Desai:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing - review & editing. **Lindsay Bottoms:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing - review & editing. **Samantha Hughes:** Conceptualization, Methodology, Formal analysis, Resources, Visualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Michelle Severins, Chiara Pignato, Inge Vrinds and Ellen Obster for their support in the experiments at the HAN University of Applied Sciences.

Author contributions

Conceptualization; SH, MR, LB. Investigation; DvdK, AvdE, JW, TD, SH. Methodology; DvdK, SH, TD, LB. Formal Analysis; SH, TD, MR, LB. Funding Acquisition; TD, LB, MR. Resources; SH, TD, LB, MR. Writing; SH, TD, MR, LB.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.103890.

References

Ashrafi, K., Chang, F. Y., Watts, J. L., Fraser, A. G., Kamath, R. S., Ahringer, J., & Ruvkun, G. (2003). Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Letters to Nature, 421*, 268–272.

Avery, L. (1993). The genetics of feeding in *Caenorhabditis elegans*. *Genetics, 133*(4), 897–917.

Bell, P. G., Gaze, D. C., Davison, G. W., George, T. W., Scotter, M. J., & Howatson, G. (2014). Montmorency tart cherry (*Prunus cerasus L.)* concentrate lowers uric acid, independent of plasma cyanidin-3-O-glucosiderutinoside. *Journal of Functional Foods, 11*, 82–90.

Bell, P. G., McHugh, M. P., Stevenson, E., & Howatson, G. (2014). The role of cherries in exercise and health. *Scandinavian Journal of Medicine & Science in Sports, 24*(3), 477–490.

Bell, P. G., Stevenson, E., Davison, G. W., & Howatson, G. (2016). The effects of montmorency tart cherry concentrate supplementation on recovery following prolonged. *Intermittent Exercise. Nutrients, 8*(7), e441.

Blando, F., Gerardi, C., & Nicoletti, I. (2004). Sour cherry (*Prunus cerasus L.)* Anthocyanins as ingredients for functional foods. *Journal of Biomedicine and Biotechnology, 4*, 253–258.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics, 77*, 71–94.

Buchter, C., Ackermann, D., Havermann, S., Honnen, S., Chovolou, Y., Fritz, G., ... Watjen, W. (2013). Myricetin-mediated lifespan extension in *Caenorhabditis elegans* is modulated by DAF-16. *International Journal of Molecular Sciences, 14*(6), 11895–11914.

Chai, S. C., Davis, K., Zhang, Z., Zha, L., & Firschner, K. F. (2019). Effects of tart cherry juice on biomarkers of imflammation and oxidative stress in older adults. *Nutrients, 11*(2), 228.

Chang, W.-H., Hu, S.-P., Huang, Y.-F., Yeh, T.-S., & Liu, J.-F. (2010). Effect of purple sweet potato leaves consumption on exercise-induced oxidative stress and IL-6 and HSP72 levels. *Journal of Applied Physiology, 109*, 1710–1715.

Chen, W., Muller, D., Richling, E., & Wink, M. (2013). Anthocyanin-rich purple wheat prolongs the lifespan of *Caenrohaditis elegans* probably by activating the DAF-16/FOXO transcription factor. *Journal of Agricultural and Food Chemistry, 61*, 3407–13053.

Corton, J. C., Apte, U., Anderson, S. P., Limaye, P., Yoon, L., Latendresse, J., ... Mehendale, H. M. (2004). Mimetics of caloric restriction include agonists of lipid-activated nuclear receptors. *J Biol Chem, 279*(44), 46204–46212. https://doi.org/10.1074/jbc.M406739200.

Dansen, T. B., Kops, G. J. P. L., Denis, S., Jelluma, N., Wanders, R. J. A., Bos, J. L., ... Wirtz, K. W. A. (2004). Regulation of steril carrier protein gene expression by the Forkhead transcription factor FOXO3a. *The Journal of Lipid Research, 45*, 81–88.

Desai, T., Bottoms, L., & Roberts, M. (2018). The effects of Montmorency tart cherry juice supplementation and FATMAX exercise on fat oxidation rates and cardio-metabolic markers in healthy humans. *European Journal of Applied Physiology, 118*(2), 2535–2539.

Desai, T., Roberts, M., & Bottoms, L. (2019). Effects of Montmorency tart cherry supplementation on cardio-metabolic markers in metabolic syndrome participants: A pilot study. *Journal of Functional Foods, 57*, 286–298.

Ferreira, L. F., & Behnke, B. J. (2010). A toast to health and performance! Beetroot juice lowers blood pressure and the O2 cost of exercise. *Journal of Applied Physiology, 110*, 585–586.

Gao, C., Gao, Z., Greenway, F. L., Burton, J. H., Johnson, W. D., Keenan, M. J., ... Zheng, J. (2015). Oat consumption reduced fat deposostion and improved health span in *Caenorhabditis elegans* model. *Nutritional Research, 35*(9), 834–843.

Ghosh, D. (2005). Anthocyanins and anthocyanin-rich extracts in biology and medicine: Biochemical, cellular, and medicinal properties. *Current Topics in Nutraceutical Research, 3*(2), 113–124.

Greer, E. L., & Brunet, A. (2009). Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. *Aging Cell, 9*(2), 113–127.

Grunz, G., Haas, K., Soukup, S., Klingenspor, M., Kulling, S. E., Daniel, H., & Spanier, B. (2012). Structural features and bioavailability of four flavanoids and their implications for lifespan-extending and antioxidant actions in *C. elegans*. *Mechanisms of Ageing and Development, 133*(1), 1–10.

Hansen, M., Flatt, T., & Aguilaniu, H. (2013). Reproduction, fat metabolism and life span: What is the connection? *Cell Metabolism, 17*(1), 10–19.

Hashmi, S., Wang, Y., Parhar, R. S., Collison, K. S., Conca, W., Al-Mohanna, F., & Gaugler, F. (2013). A *C. elegans* model to study human metabolic regulation. *Nutrition and Metabolism, 10*, 31–42.

Hiller, L. W., Coulson, A., Murray, J. I., Bao, Z., Sulston, J. E., & Waterson, R. H. (2005). Genomics in *C. elegans*: So many genes, such a little worm. *Genome Research, 15*(12), 1651–1660.

Holubkova, A., Penesova, A., Sturdik, E., Mosovska, S., & Mikusova, L. (2012). Phytochemicals with potential effects in metabolic syndrome prevention and therapy. *Acta Chimica Slovaca, 5*(2), 186–199.

Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., van Someren, K. A., Shave, R. E., & Howatson, S. A. (2010). Influence of tart cherry juice on indices of recovery following marathon running. *Scandinavian Journal of Medicine & Science in Sports, 20*(6), 843–852.

Johnson, S. A., Navaei, N., Pourafshar, S., Jaime, S. J., Akhavan, N. S., Alvarez-Alvarado, S., ... Figueroa, A. (2017). Effects of tart cherry juice on brachial and aortic hemodynamics, arterial stiffness, and blood biomarkers of cardiovascular health in adults with metabolic syndrome. *The FASEB Journal, 31*.

Jowko, E., Sacharuk, J., Balasinska, B., Ostaszewski, P., Charmas, M., & Charmas, R. (2011). Green tea extract supplementation gives protection against exercsie-induced oxidatitve damage in healthy men. *Nutritional Research, 31*, 813–821.

Kaletta, T., & Hengartner, M. O. (2006). Finding function in novel targets: *C. elegans* as a model organism. *Nature Reviews Drug Discovery, 5*, 387–398.

Kamath, R. S., & Ahringer, J. (2003). Genome-wide screening in *Caenorhabditis elegans*. *Methods, 30*(4), 313–321.

Keane, K. M., Bell, P. G., Lodge, J. K., Constantinou, C. L., Jenkinson, S. E., Bass, R., & Howatson, G. (2016). Phytochemical uptake following human consumption of Montmorency tart cherry (*Prunus cerasus* L.) and influence of phenolic acids on vascular smooth muscle cells *in vitro*. *European Journal of Nutrition, 55*(4), 1695–1705.

Keane, K. M., George, T. W., Constantinou, C. L., Brown, M. A., Clifford, T., & Howatson, G. (2016). Effects of Montmorency tart cherry (*Prunus Cerasus L.*) consumption on vascular function in men with early hypertension. *The American journal of clinical nutrition, 103*(6), 1531–1539.

Keane, K. M., Haskell-Ramsey, C. F., Veasey, R. C., & Howatson, G. (2016). Montmorency Tart cherries (*Prunus cerasus* L.) modulate vascular function acutely, in the absence of improvement in cognitive performance. *British Journal of Nutrition, 116*(11), 1935–1944.

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthicyanins: Coloured pogments as food, pharmaceutical ingredients and the potential health benefits. *Food and Nutritional Research, 61*(1), 1361779.

Kirakosyan, A., Gutierrez, E., Solano, B. R., Seymour, E. M., & Bolling, S. F. (2018). The inhibitory potential of Montmorency tart cherry on key enzymes relevant to type 2 diabtetes and cardiovascular disease. *Food Chemistry, 252*, 142–146.

Kirakosyan, A., Seymour, E. M., Urcuyo-Lianes, D. E., Kaufman, P. B., & Bolling, S. F. (2009). Chemical profile and antioxidant capacities of tart cherry products. *Food Chemistry, 115*(1), 20–25.

Kirakosyan, A., Seymour, E. M., Wolforth, J., McNish, R., Kaufman, P. B., & Bolling, S. F. (2015). Tissue bioavailablity of anthocyanins from whole tart cherry in healthy rats. *Food Chemistry, 171*(15), 26–31.

Kock, K., Weldle, N., Baler, S., Buchter, C., & Watjen, W. (2019). *Hibiscus sabdariffa* L. extract prolongs lifespan and protects against amyloid-β toxicity in *Caenorhabditis elegans*: Involvement of the FoxO and Nrf2 orthologues DAF-16 and SKN-1. *European Journal of Nutrition*.

Konczak, I., & Zhang, W. (2004). Anthocyanins - More than natures colours. *Journal of Biomedicine and Biotechnology, 5*, 239–240.

Lakowski, B., & Hekimi, S. (1998). The genetics of caloric restriction in *Caenorhabditis elegans*. *PNAS, 95*(22), 13091–13096.

Laranjeiro, R., Harinath, G., Burke, D., Braeckman, B. P., & Driscoll, M. (2017). Single swim sessions in *C. elegans* induce key features of mammalian exercise. *BMC Biology, 15*(30), 1–17.

Lehner, B., Crombie, C., Tischler, J., Fortunato, A., & Fraser, A. G. (1996). Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. *Nature Genetics, 38*(8), 896–903.

Lesanpezeshki, L., Hewitt, J. E., Laranjeiro, R., Antebi, A., Driscoll, M., Szewczyk, N. J., ... Vanapalli, S. A. (2019). Pluronic gel-based burrowing assay for rapid assessment of neuromuscular health in *C. elegans*. *Scientific Reports, 9*, 15246.

Levers, K., Dalton, R., Galvan, E., Goodenough, C., O'Conner, A., Simbo, S., ... Kreider, R. B. (2015). Effects of powdered Montmorency tart cherry supplementation on an acute bout of intense lower body strength exercise in resistance trained males. *Journal of the International Society of Sports Nutrition, 41*.

Levers, K., Dalton, R., Galvan, E., O'Conner, A., Goodenough, C., Simbo, S., ... Krieder, R. B. (2016). Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals. *Journal of the International Society of Sports Nutrition, 13*, 22.

Lynn, A., Mathew, S., Moore, C. T., Russell, J., Robinson, E., Soumpasi, V., & Barker, M. E. (2014). Effect of a tart cherry juice supplement on arterial stiffness and inflammation in healthy adults: A randomised controlled trial. *Plant Foods for Human Nutrition, 69*(2), 122–127.

Mak, H. Y., Nelson, L. S., Basson, M., Johnson, C. D., & Ruvkun, G. (2006). Polygenic control of *Caenorhabditis elegans* fat storage. *Nature Genetics, 38*(3), 363–368.

Markaki, M., & Tavernarakis, N. (2010). Modeling human diseases in *Caenorhabditis elegans*. *Biotechnology Journal, 5*(12), 1261–1276.

McAnulty, L. S., Nieman, D. C., Dumke, C. L., Shooter, L. A., Henson, D. A., Utter, A. C., ... McAnulty, S. R. (2011). Effect of blueberry ingestion on natural killer cell counts, oxidative stress and inflammation prior to and after 2.5 h of running. *Applied Physiology and Nutritional Metabolism, 36*, 976–984.

Mejia-Martinez, F., Franco-Juarez, B., Moreno-Arriola, E., Hernandez-Vazquez, A., Martinez-Avila, M., Gomez-Manzo, S., ... Ortega-Cuellar, D. (2017). The MXL-3/SBP-1 axis is responsible for glucose-dependent fat accumulation in *C. elegans*. *Genes, 8*(307), 1–15.

Nunn, A. V. W., Bell, J., & Barter, P. (2007). The integration of lipid-sensing and anti-inflammatory effects: How the PPARs play a role in metabolic balance. *Nuclear Receptor, 5*, 1–13.

O'Neill, S., Bohl, M., Gergersen, S., Hermansen, K., & O'Driscoll, L. (2016). Blood-based biomarkers for metabolic syndrome. *Trends in Endrocrinology and Metabolism, 27*(6), 363–374.

O'Rourke, E. J., Soukas, A. A., Carr, C. E., & Ruvkin, G. (2009). *C. elegans* major fats are stored in vesicles distinct form lysosome-related organelles. *Cellular Metabolism, 10*(5), 430–435.

Ou, B., Bosak, K. N., Brickner, P. R., Iezzoni, D. G., & Seymour, E. M. (2012). Processed tart cherry products-comparative phytochemical content, *in vitro* antioxidant capacity and in vitro anti-inflammatory activity. *Journal of Food Science, 77*(5), H105–H112.

Pang, S., Lynn, D. A., Lo, J. Y., Paek, J., & Curran, S. P. (2014). SKN-1 and Nrf2 couples proline catabolism with lipid metabolism during nutrient deprivation. *Nature Communications, 5*, 5048. https://doi.org/10.1038/ncomms6048.

Reis, J. F., Monteiro, V. V. S., de Souza Gomes, R., de Carmo, M. M., de Costa, G. V., Ribera, P. C., & Monterio, M. C. (2016). Action mechanism and cardiovascular effect of anthocyanins: A systematic review of animal and human studies. *Journal of Translational Medicine, 14*, 315.

Rual, J. F., Caron, J., Koreth, J., Hao, T., Noicot, A. S., Hirozane-Kishikawa, T., ... Vidal, M. (2004). Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. *Genome Research, 14*(10B), 2162–2168.

Saklayen, M. G. (2018). The global epidemic of the metabolic syndrome. *Current Hypertension Reports, 20*(2), 12.

Scazzocchio, B., Varì, R., Filesi, C., D'Archivio, M., Santangelo, C., Giovannini, C., ... Masella, R. (2011). Cyanidin-3-O-β-glucoside and protocatechuic acid exert insulin-like effects by upregulating PPARγ activity in human omental adipocytes. *Diabetes, 60*(9), 2234–2244.

Seymour, E. M., Lewis, S. K., Urcuyo-Lianes, D. E., Tanone, I. I., Kirakosyan, A., Jaufman, P. B., & Bolling, S. F. (2009). Regular tart cherry intake alters abdominal adiposity, adipose gene transcription, and inflammation in obesity-prone rats fed a high fat diet. *Journal of Medicinal Food, 12*(5), 935–942.

Seymour, E. M., Singer, A. A., Kirakosyan, A., Urcuyo-Lianes, D. E., Kaufman, P. B., & Bolling, S. F. (2008). Altered hyperlipidemia, hepatic steatosis, and hepatic peroxisome proliferator-activated receptors in rats with intake of tart cherry. *Journal of Medicinal Food, 11*(2), 252–259.

Seymour, E. M., Warber, S. M., Kirakosyan, A., Noon, K., Gillespie, B., Uhley, V. E., ... Bolling, S. F. (2014). Anthocyanin pharmacokinetics and dose-dependent plasma antioxidant pharmacodynamics following whole tart cherry intake in healthy humans. *Journal of Functional Foods, 11*(C), 509–516.

Shaye, D. D., & Greenwald, I. (2011a). OrthoList: A compendium of *C. elegans* genes with human homologs. *PLoS One, 9*, 10.

Shaye, D. D., & Greenwald, I. (2011b). OrthoList: A compendium of *C. elegans* genes with human orthologs. *PLOS One, 9*(1), 10.

Stiernagle, T. (2006). Maintenance of C. elegans (D. Fay Ed.).

Sulston, J. E., & Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. *Developmental Biology, 56*(1), 110–156.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*, 63–68.

Tahsini, L., & Heydari, R. (2012). Anti-diabetic effect of cherries in alloxan induced diabetic rats. *Recent Patents on Endocrine, Metabolic and Immune Drug Discovery, 6*, 67–72.

Tambara, A. L., de Los Santos, L., Moraes, Dal Forno, A. H., Boldori, J. R., Goncalves, S. A. T., de Freitas Rodrigues, C., ... Denardin, C. C. (2018). Purple pitanga fruit (Eugenia uniflora L.) protects against oxidative stress and increase the lifespan in Caenorhabditis elegans via the DAF-16/FOXO pathway. *Food and Chemical Toxicology, 120*, 639–650.

Thrombold, J. R., Reinfeld, A. S., Casler, J. R., & Coyle, E. F. (2011). The effect of pomegranate juice supplementation on strength and soreness after eccentric exercise. *Journal fo Strength and Conditioning Research, 25*, 1782–1788.

Traustadottir, T., Davies, S. S., Stock, A. A., Su, Y., Hewerd, C. B., Roberts, L. J. I., & Harman, S. M. (2009). Tart cherry juice decreases oxidative stress in healthy older men and women. *Journal of Nutrtition, 139*, 1896–1900.

van Gilst, M. R., Hadjivassiliou, H., Jolly, A., & Yamamoto, K. R. (2005). Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composistion in *C. elegans*. *PLoS Biology, 3*(2), e53.

van Heemst, D. (2010). Insuin, IGF-1 and longevity. *Aging and Disease, 1*(2), 147–157.

Wang, L.-S., & Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters, 269*, 281–290.

Wilson, M. A., Shukitt-Hale, B., Kalt, W., Ingram, D. K., Joseph, J. A., & Wolkow, C. A. (2006). Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. *Aging Cell, 5*(1), 59–68.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, S. E., Gebhardt, R. L., & Prioir, R. L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry, 54*(11), 4069–4075.

Xiong, H., Pears, C., & Woollard, A. (2017). An enhanced *C. elegans* based platform for toxicity assessment. *Scientific Reports, 7*(1), 9839.

Yang, J. S., Nam, H. J., Seo, M., Han, S. K., Choi, Y., Nam, H. G., ... Kim, S. (2011). OASIS: Online Application for the Survival Analysis of Lifespan Assays Performed in Aging Research. *PLoS One, 6*(8), e23525.