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Arthrospira platensis F&M-C265 reduces cardiometabolic risk factors in rats fed a high fat diet

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ABSTRACT

Clinical studies indicate that *Arthrospira platensis (A. platensis)* mitigates cardiometabolic risk factors, but the underlying mechanisms are very elusive. To fill this gap, Sprague-Dowley rats were fed either AIN-76 normal diet (5 % corn oil) or high-fat (HF; 30 % lard + 3 % corn oil) or HF + 5 % *A. platensis* F&M-C256 diet for 3 months. *A. platensis* F&M-C256 decreased blood triglycerides and total cholesterol, systolic and diastolic pressure and enhanced the expression of thermogenesis-related genes Prdm16, Dio2, PPARγ, Ucp1 and Lpl in visceral adipose tissue, compared to HF-diet. *A. platensis* reduced ANGPTL3 plasma levels and hepatic steatosis, prevented periaortic adipose tissue hypertrophy and increased aortic eNOS expression. These data provide some mechanistic evidence about the beneficial effects of *A. platensis* observed in human studies. Further controlled trials are needed to verify the clinical usefulness of *A. platensis* supplementation against metabolic disorders, to select the dosing regimens and the subgroup of patients with likelihood of benefit.

1. Introduction

Metabolic syndrome (MetS) defines a group of cardiometabolic risk factors including abdominal obesity, impaired glucose tolerance, hypertriglyceridemia, decreased high-density lipoprotein (HDL) cholesterol, and hypertension (Huang, 2009). Nowadays, MetS reached "pandemic" proportions, affecting a quarter of the world's population and feeds into the risk of diabetes and cardiovascular disease (CVD) morbidity and mortality (Saklayen, 2018). Due to the coexistence of interrelated risk factors, the management of MetS involves a dual approach that combines lifestyle/dietary changes and multi-pharmacological interventions with lipid lowering, anti-hypertensive and anti-diabetic therapies (Rochlani et al., 2017).

Adipose tissue is a complex organ involved in the regulation of energy homeostasis and a key player in the development of metabolic disorders. While the main function of white adipose tissue (WAT) is the storage of energy in the form of triglycerides, brown adipose tissue (BAT) uses nutrients to produce heat in a process known as adaptive thermogenesis (Cho et al., 2023). A potential tool against cardiometabolic disorders is the promotion of WAT browning which generally involves the activation of β3-Adrenergic Receptor/ Uncoupling protein 1 (Ucp1) signaling (D'Ambrosio et al.,2023). However, other mediators are emerging, including angiopoietin-related protein3 (ANGPTL3), a secreted hepatokine that inhibits lipoprotein lipase (Lpl) activity and uptake of circulating triglycerides in adipose tissue (Banfi et al., 2018) and also suppresses Ucp1 expression and WAT browning

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(Lv et al., 2022).

Improving energy metabolism through dietary factors capable of evoking WAT browning, represents a promising strategy against metabolic disorders (Okla et al., 2017; Jia et al., 2023). Microalgae have received increasing attention as new sources of bioactive compounds for controlling metabolic disturbances (Tamel Selvan et al., 2023). *Arthrospira platensis (A. platensis)*, formerly known as *Spirulina platensis* or simply, spirulina, is the most popular microalga in the European Union and it is considered GRAS (Generally Recognized as Safe) for human consumption (Sokary et al., 2024; Niccolai et al., 2017). In virtue of its well-known nutritional profile including macro and micronutrients (proteins, γ-linolenic acid, vitamins, and minerals) and other bioactive compounds such as C-phycocyanin, *A. platensis* is widely commercialized as a functional food supplement or nutraceutical (Kulshreshtha et al., 2008). We reported that a diet supplemented with high dose (20 %) of *A. platensis* F&M-C256 biomass, positively affected lipid homeostasis even in a balanced dietary regimen (Bigagli et al., 2017). The effects of *A. platensis* on risk factors associated with MetS are increasingly documented and meta-analysis of clinical trials provided substantial evidence that spirulina supplementation has favorable effects on cardiometabolic biomarkers in humans, including lipid, glucose, and blood pressure (Rahnama et al., 2023; Hamedifard et al., 2019; Machowiec et al., 2021). Results from another recent meta-analysis demonstrated high certainty of evidence that spirulina supplementation resulted in a small weight loss in adults with overweight or obesity (Shahinfar et al., 2023). A recent randomized, double-blind placebo-controlled trial also demonstrated that *A. platensis* supplementation as an add-on to metformin, significantly improved blood glucose and lipids in patients with inadequately controlled type 2 diabetes mellitus (Karizi et al., 2023).

Despite the growing body of evidence indicating positive effects of *A. platensis* on cardiometabolic health, the underlying mechanisms remain largely unknown. In this study, the beneficial effects of dietary supplementation with 5 % *A. platensis* F&M-C256 (4 g/kg bw/d) against cardiometabolic risk factors of MetS were therefore investigated in rats fed high-fat diet; the involved molecular mechanisms were explored by focusing on adipose tissue, a key endocrine regulator of metabolic homeostasis.

2. Materials and methods

2.1. Algal biomass production and composition

A. platensis F&M-C256, from the Fotosintetica & Microbiologica S.r.l. Culture Collection of Microalgae and Cyanobacteria, was cultivated in GWP® photobioreactors as previously reported (Tredici et al., 2011) in a semi-batch mode, in Zarrou medium. Its original geographical region is alkaline lake, Chad.

2.2. Diet preparation

Control AIN-76 (NF), High-Fat (HF) and *A. platensis* F&M-C256 supplemented High-Fat (HFA) diets were prepared using components purchased from Piccioni Laboratories (Gessate, Milan, Italy) and a mechanical mixer. As reported in bold in Supplementary Table 1, in the microalga-supplemented diet containing 5 % lyophilized *A. platensis* F&M-C256, the content of proteins, lipids, carbohydrates, and fibers was adjusted according to the amounts of these components in the microalgal biomass, as we previously reported (Bigagli et al., 2017). The AIN76 diet provided 3.79 kcal/g, while HF and HFA diets provided both 5.33 kcal/g.

2.3. Animals and treatment design

Animal care and experimental procedures carried out in compliance with the guidelines of the Italian Ministry of Health (Decree Law n. 26/2014), following the European directive 2010/63/UE and the protocol was approved (authorization n. 1137/2015-PR) by the national Committee set by the Italian Ministry of Health. All animal procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0.

After 1 week of acclimatization in plastic cages, male Sprague-Dowley rats (Nossan S.r.l., Milan, Italy), aged 6–8 weeks, were divided in three experimental groups: rats fed AIN-76 diet (NF, 5 % of fat; n = 6), rat fed High Fat Diet (HF, 30 % of fat; n = 6) and rat fed a HF diet supplemented with 5 % *A. platensis* F&M-C256 biomass (HFA, 30 % of fat; n = 6). Water and food were available *ad libitum*; the lighting regime was a standard 12 h light and 12 h dark, temperature was maintained constant at 21 ± 2 °C. Food and water consumption were monitored every two days throughout the entire experiment.

At the end of the study, after 3 months feeding, rats were euthanized by inhalation of CO_2 and blood samples were collected in tubes containing an anticoagulant (Ethylenediaminetetraacetic acid - EDTA) to separate plasma. Following the sacrifice, liver and visceral, epididymal and renal adipose tissue were weighed and stored either at – 20 °C or – 80 °C for further analyses.

2.4. Blood pressure measurement

Systolic and diastolic blood pressure were measured in conscious rats by a computerized, non-invasive, tail-cuff method (Visitech BP-2000 Series II Blood Pressure Analysis System, USA). The same researcher performed all measurements, repeated five times, in a quiet environment; the highest and lowest values were discarded. Values were reported as mean ± standard error of mean (SEM).

2.5. Histological analyses

For histological analyses, tissue samples were stored in neutral formaldehyde and embedded in paraffin. Histological sections of 5 μm were stained with hematoxylin and eosin. Microscopic analysis was performed with the ACT-2U software program (Nikon, Instruments Europe, Badhoevedorp, The Netherlands) connected via a camera to the microscope (Optiphot-2; Nikon). Hepatic fat content was evaluated as steatosis score as previously reported (D'Ambrosio et al., 2023). The periaortic adipocyte area was calculated using ImageJ software (NIH): adipocyte cross-sectional area of 300 fat cells per group was obtained from perimetral tracings of all adipocytes within a field of view.

2.6. Blood biochemistry

Plasma levels of total Cholesterol (TC), triglycerides (TGs) and glucose were measured with the Reflotron® Plus system (Roche Diagnostics GmbH, Mannheim, Germany). ANGPTL3 plasma levels were measured using the immunoenzymatic method ANGPTL3 (mouse/rat) Dual ELISA Kit (AdipoGen® Life Science, Fuellinsdorf, Switzerland) according to the manufacturer's specifications.

2.7. Fecal lipid content

Fecal lipid content was measured from dried fecal samples (about 30 mg) according to Kraus et al. (2015).

2.8. Reverse transcription-polymerase chain reaction

Total RNA from visceral adipose tissue was extracted using the TRIzol reagent (Invitrogen, Thermo Fisher Scientific, US), according to the manufacturer's specifications. For first-strand cDNA synthesis, 100 ng of total RNA from each sample was reverse-transcribed using the Revert Aid RT Kit (Thermo Fisher Scientific, US). Primers were designed based on the rat GenBank sequences (2. For each target gene, the relative amount of mRNA in the samples was calculated as the ratio of each gene

to β-Actin mRNA (Luceri et al., 2017). The STRING database (https://string-db.org/) was used to construct the gene interaction network of Ucp1 in rodents.

2.9. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8.0.2 software (GraphPad, San Diego, CA). D'Agostino and Pearson omnibus normality test was applied to verify the Gaussian distribution of each variable. Differences on body and organs weight, clinical chemistry parameters, fecal lipid content, blood pressure measurements and on gene expression were analyzed using one-way ANOVA and Tukey's multiple comparisons test. Results are expressed as means ± SEM. Significance was assigned at $p < 0.05$.

3. Results

3.1. Effects of A. platensis F&M-C256 on body and organs weight

As shown in Fig. 1, HF diet moderately increased body weight compared to NF diet and no significant variations were observed in rats fed *A. platensis* F&M-C256 compared to HF that also had similar food intake (data not shown). Similarly, *A. platensis* F&M-C256 did not counteracted HF diet induced increase in visceral, epididymal and renal fat but reduced liver weight ($p < 0.05$).

3.2. Effects of A. platensis F&M-C256 on plasma lipids, glucose and on blood pressure

HF diet did not induce a significant increase in plasma TG, TC and glucose compared to NF diet. However, *A. platensis* F&M-C256 supplementation reduced TG and TC levels compared to HF diet ($p < 0.05$ and < 0.01, respectively). SBP and DBP were also not affected by HF diet but *A. platensis* F&M-C256 enriched diet significantly reduced these parameters not only compared to the HF diet, but also to the NF diet group (Table 1).

3.3. Effects of A. platensis F&M-C256 on thermogenesis-related genes in visceral WAT

To explore the mechanisms involved in the beneficial effects of *A. platensis* F&M-C256 on TC and TG, we analyzed the expression of thermogenesis-related genes in visceral WAT. As shown in Fig. 2A, HF diet did not modify the expression of β3-Adrenergic receptor (Adrb3), type 2 deiodinase (Dio2) and uncoupling protein-1 (Ucp1) genes and slightly reduced the expression of peroxisome proliferative-activated receptor gamma (Pparγ) compared to NF. *A. platensis* F&M-C256-diet induced the expression of PR domain containing 16 (Prdm16, $p < 0.001$), a gene not expressed in visceral WAT from both NF and HF diet groups. Compared to HF, an increased expression of Dio2 ($p < 0.05$), Pparγ ($p < 0.01$) and Ucp1 ($p < 0.01$) genes was also observed in *A. platensis* F&M-C256 fed rats, while the expression of β3 receptor was unaffected.

Table 1
Effects of *A. platensis* F&M-C256 on plasma lipids, glucose, and blood pressure.

	NF	HF	HFA
Triglycerides (mg/dl)	205 ± 25.06	258 ± 55.43	155 ± 12.62 *
Total Cholesterol (mg/dl)	127.4 ± 3.91	134 ± 6.14	107 ± 3.45 **
Glucose (mg/dl)	169 ± 8.80	211 ± 10.64	198 ± 21.77
SBP (mm Hg)	158 ± 3.76	160 ± 2.46	136 ± 4.28 ^,**
DBP (mm Hg)	91 ± 4.05	112 ± 3.06	73 ± 3.92 ^, ***

Data are expressed as mean ± SEM (n = 6). SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure). Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + 5 % of *A. platensis* F&M-C256 (HFA).^$p < 0.05$ vs NF; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs HF by one-way ANOVA and Tukey's multiple comparisons test.

By using STRING analysis software, we highlighted the interaction networks among the genes significantly modulated by *A. platensis* F&M-C256 and related metabolic pathways: Thermogenesis (green spot), Diet induced thermogenesis (red spot), Brown fat cell differentiation (blue spot), Regulation of lipid storage (yellow spot) and Regulation of metabolic process (pink spot) (Fig. 2B).

3.4. Effects of A. platensis F&M-C256 on ANGPTL3-Lpl axis

A. platensis F&M-C256 diet reduced ANGPTL3 plasma levels compared to HF ($p < 0.001$). This reduction was associated to a significant increase in Lpl gene expression in visceral WAT of rats fed *A. platensis* F&M-C256 diet compared to HF rats ($p < 0.05$). Fig. 3.

3.5. Effects of A. platensis F&M-C256 on hepatic steatosis and fecal lipids content

Histological liver analysis showed that HF diet induced an increase in the steatosis score compared to NF diet group ($p < 0.001$) and that *A. platensis* F&M-C256-diet counteracted this effect ($p < 0.05$). Moreover, despite not statistically significant, an increased fecal lipid excretion was observed in *A. platensis* F&M-C256 group compared to HF. Fig. 4.

3.6. Effects of A. platensis F&M-C256 on periaortic adipose tissue

Histological analysis of periaortic adipocytes showed that HF diet

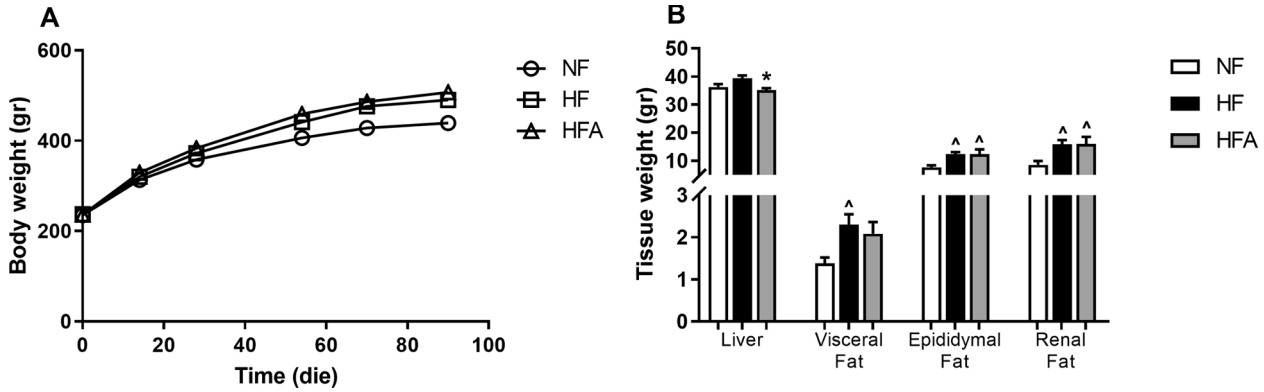

Fig. 1. A. Effects of experimental diets on body weight. **B.** Organ weights (liver, visceral, epididymal and renal fat). Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + 5 % of *A. platensis* F&M-C256 (HFA). Data are expressed mean ± SEM; n = 6 rats/group.^$p < 0.05$ vs NF, * $p < 0.05$ vs HF, by one-way ANOVA and Tukey's multiple comparisons test.

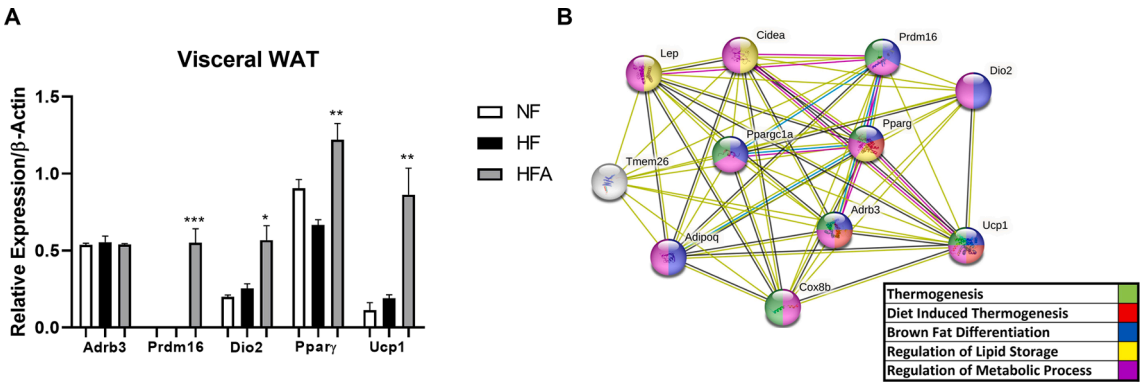

Fig. 2. A. Expression of thermogenesis-related genes in visceral WAT. β3-Adrenergic receptor (Adrb3), PR domain containing 16 (Prdm16), type 2 deiodinase (Dio2), peroxisome proliferative-activated receptor gamma (Pparγ) and uncoupling protein-1 (Ucp1). **B.** Interaction networks of genes significantly modulated by *A. platensis* F&M-C256 in visceral WAT identified by using STRING analysis software. Data are expressed as mean ± SEM; n = 6 rats/group. Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + 5 % of *A. platensis* F&M-C256 (HFA). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs HF, by one-way ANOVA and Tukey's multiple comparisons test.

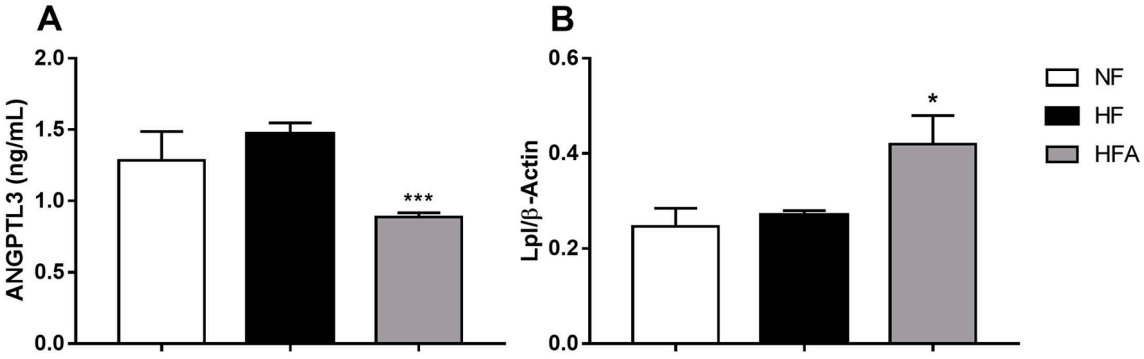

Fig. 3. A. ANGPTL3 plasma levels. **B.** Lipoprotein lipase (Lpl) expression in visceral WAT. Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + 5 % of *A. platensis* F&M-C256 (HFA). Data are expressed as mean ± SEM; n = 6 rats/group. * $p < 0.05$ and *** $p < 0.001$ vs HF by one-way ANOVA and Tukey's multiple comparisons test.

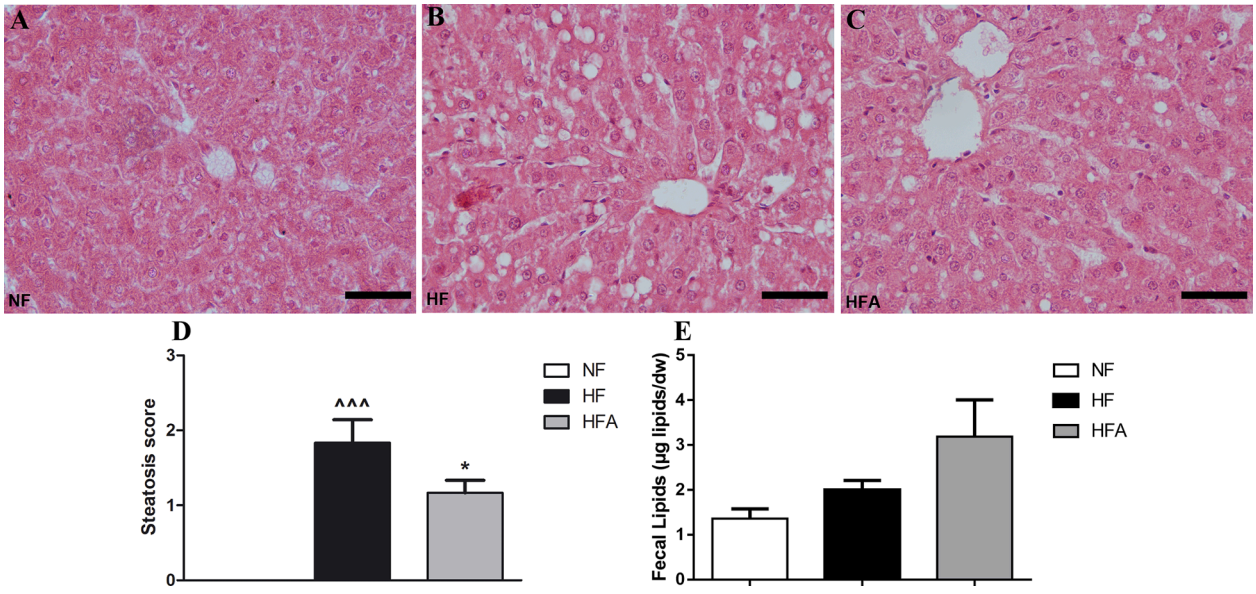

Fig. 4. A-C. Hematoxylin and eosin staining for histopathological analysis of rat hepatic tissues in the different groups at 400X magnification. **A.** NF group; **B.** HF group; **C.** HFA group. **D.** Steatosis score. **E.** Mean fecal lipid content. Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + 5 % of A. platensis F&M-C256 (HFA). Data are expressed as mean ± SEM; n = 6 rats/group.^^^p < 0.001 vs NF and * $p < 0.05$ vs HF, by one-way ANOVA and Tukey's multiple comparisons test.

induced adipocytes hypertrophy as demonstrated by the increase of the adipocytes area compared to NF diet group ($p < 0.001$) and that this effect was completely prevented by *A. platensis* F&M-C256 ($p < 0.001$). Moreover, the phenotype of adipocytes from *A. platensis* F&M-C256 group resembled that of brown adipocytes (smaller cells with a centrally located nucleus and many small lipid droplets, white arrows). Fig. 5.

3.7. Effect of A. platensis F&M-C256 on aortic eNOS expression

To confirm the ability of *A. platensis* F&M-C256 rich diet to activate a thermogenic program, we explored the involvement of endothelial Nitric Oxide Synthase (eNOS)-dependent vasodilation as a possible mechanism underlying the observed reduction of blood pressure. As reported in Fig. 6, in the aorta, we observed a slight, not statistically significant reduction of eNOS expression in HF-diet fed rats compared to NF-diet groups. On the contrary, *A. platensis* F&M-C256 rich diet increased the expression of eNOS ($p < 0.01$).

4. Discussion

In this work, we demonstrated the positive effects of a diet enriched with *A. platensis* F&M-C256 biomass on some metabolic alterations induced by a diet rich in fat (30 % lard + 3 % corn oil). These effects were associated to visceral WAT browning, the metabolic shift of visceral WAT into a beige/brite phenotype. In the visceral WAT of *A. platensis* F&M-C256 fed rats, we in fact observed the up-regulation of the main thermogenesis-related genes Prdm16, Dio2, Pparγ and Ucp1. Prdm16 is a pivotal regulator of beige/brite adipocytes induction (browning) and maintenance; beige/brite adipocytes may in fact reconvert into white adipocytes when Prdm16 expression is low or null (Cohen et al., 2014). Ucp1 is expressed in the brown adipocyte's mitochondria and, by uncoupling oxidative phosphorylation from ATP synthesis, dissipates the energy provided by TGs in the form of heat (Giordano et al., 2016). Prdm16 and Ucp1 are among the markers to identify beige/brite adipocytes within WAT (Pilkington et al., 2021). In addition, increased expression of Dio2 gene in visceral WAT can locally affect the content of the active form of thyroid hormone T3, which stimulates thermogenesis-related genes, increasing oxygen consumption and metabolic rate (de Jesus et al., 2001).

The canonical pathway to trigger thermogenic programming and lipolysis involves the activation of β3-adrenergic pathway (Tabuchi & Sul, 2021), and we recently demonstrated that the microalga *Tisochrysis lutea* promotes fat browning through β3-Adrenergic Receptor/Ucp1 signaling (D'Ambrosio et al., 2023). However, β3 receptor expression in visceral WAT of rats supplemented with *A. platensis* was not affected, suggesting the potential contribution of other mediators. Among those, ANGPTL3 is a circulating hepatokine that regulates plasma TGs and TC levels via the reversible inhibition of Lpl and mice deficient in ANGPTL3/8 showed increased fat browning and Lpl activity (Banfi et al., 2018). Owing to its Lpl inhibitory activity, targeting ANGPTL3 has also recently emerged as a novel therapeutic strategy against dyslipidemia (Malick et al., 2023). Higher plasma Lpl activity in rats fed *Spirulina* was previously reported (Iwata et al., 1990), but our data demonstrate, for the first time, that *A. platensis* reduces the circulating

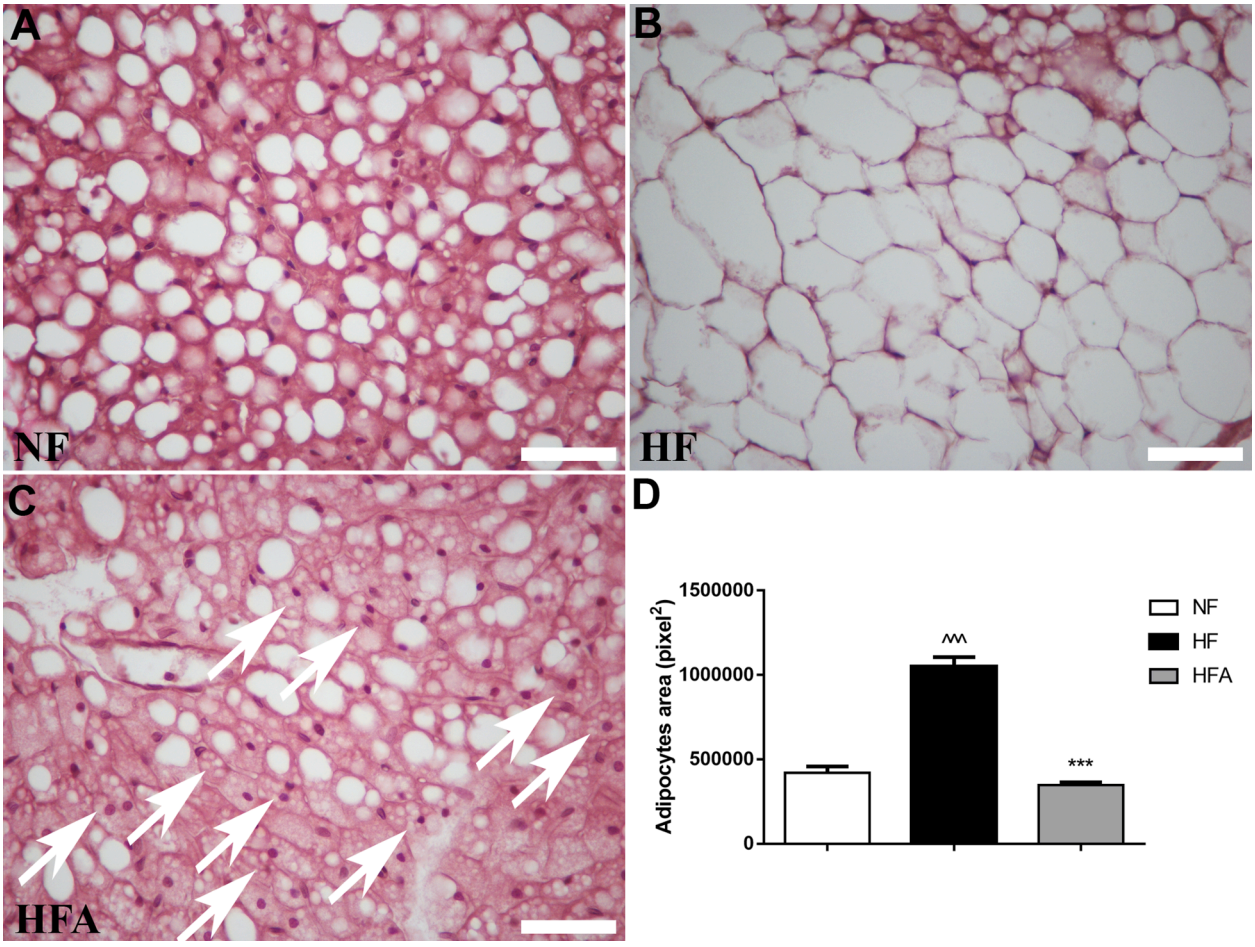

Fig. 5. Histopathological analysis of periaortic adipocytes in the different groups at 400X magnification. **A.** NF group; **B.** HF group; **C.** HFA group. **D.** Adipocytes area expressed as pixel^2. Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + 5 % of *A. platensis* F&M-C256 (HFA). Data are expressed as mean ± SEM; n = 6 rats/group.^^$p < 0.001$ vs NF and *** $p < 0.001$ vs HF, by one-way ANOVA and Tukey's multiple comparisons test.

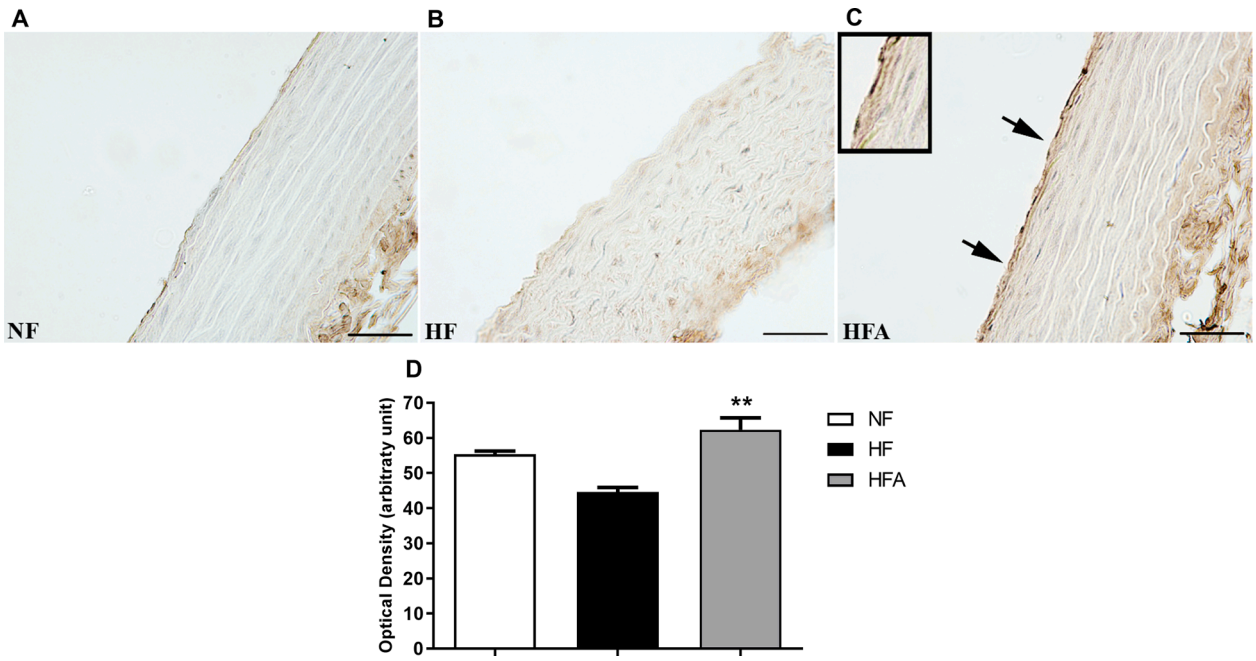

Fig. 6. Endothelial Nitric Oxide Synthase (eNOS) expression determined by immunohistochemistry (brown signal) in abdominal aortic section at 400X magnification. **A.** NF group; **B.** HF group; **C.** HFA group. **D.** densitometric analysis. The arrows point out the presence of eNOS staining in the aortic wall. Experimental groups are abbreviated as Normal Diet (NF), High Fat Diet (HF) and High Fat Diet + *A. platensis* F&M-C256 (HFA). Data are expressed as mean ± SEM; n = 6 rats/group. ** $p < 0.01$ vs HF, by one-way ANOVA and Tukey's multiple comparisons test.

pool of ANGPTL3 and that this effect is associated with increased Lpl gene expression in visceral WAT and with reduced TG and TC levels. There could be also an intriguing connection between the reduced liver steatosis, decreased ANGPTL3 plasma levels and enhanced fat browning observed in our *A. platensis* fed rats: indeed, it was demonstrated that hepatic reticulum stress, which is a common feature of steatosis, increases the secretion of ANGPTL3 that in turn binds to integrin $a_v\beta_3$ and suppresses Ucp1 through c-Jun N-terminal kinase activation in adipose tissue (Lv et al., 2022).

Various functional constituents of *A. platensis* biomass can be responsible of the observed effects; among those, phycocyanin, whose content in our *A. platensis* biomass is 8.2 % (Niccolai et al., 2019), is certainly noteworthy. In 3 T3-L1 adipocytes, an ethanolic extract of *Spirulina maxima* containing 5 % of chlorophyll *A* and 10 % of C-phycocyanin suppressed lipid accumulation by reducing the expression of key adipogenic and lipogenic proteins. In HF-fed mice, the same extract also reduced body weight gain, fat mass, TG content and TC by increasing the expression of the thermogenic factors Prdm16 and Ucp1 (Seo et al., 2018). A recent controlled, randomized, double-blind trial documented that a phycocyanin rich (883 mg/L) water extract from *A. platensis* ameliorated lipid and glucose metabolism in patients with MetS (Koite et al., 2022).

A. platensis is also a source of polyunsaturated fatty acids (PUFAs) whose content represent the 26 % of the total fat (Niccolai et al., 2019); γ-linolenic acid, which accounts for 59 % of the total PUFAs contained in *A. platensis* (Niccolai et al., 2019), increases fatty acids oxidation and Ucp1 expression (Takahashi et al., 2000). The effect of *A. platensis* F&M-C256 on ANGPTL3 may be also ascribed to its PUFAs content: indeed, a clinical trial reported that a 7-day high-PUFA diet reduced ANGPTL3 and ANGPTL8 and decreased TGs levels compared to the control diet (Kaviani et al., 2019). Nevertheless, the contribution of other bioactive compounds cannot be excluded: in fact, *A. platensis* F&M-C256 contains carotenoids such as zeaxanthin, β-cryptoxanthin and β-carotene (Bigagli et al., 2023), known to be effective in controlling key aspects of adipose tissue biology, including fatty acid oxidation, thermogenesis, and WAT browning (Tourniaire et al., 2009; Bonet et al., 2015); in 3 T3-L1 adipocytes, zeaxanthin promoted mitochondrial biogenesis and adipocyte browning by increasing Ucp1 and Prdm16 (Zhao et al., 2021) while β-cryptoxanthin repressed adipocyte hypertrophy and improved lipid profiles in obese mice (Takayanagi et al., 2011).

Moreover, *A. platensis* F&M-C256 diet prevented HF diet-induced periaortic fat hypertrophy and promoted its metabolic shift towards a brown-like phenotype. The increase whole-body energy expenditure through the activation of brown adipose tissue but also through WAT browning, promotes energy dissipation through heat release, thus producing vasodilation (Thoonen et al., 2016). Accordingly, *A. platensis F&M-C256*−fed rats showed reduced blood pressure and increased eNOS expression in the aortic wall. The relevance of thermogenesis activation and eNOS expression for the control of blood pressure has been extensively reported (van Haperen et al., 2002; Saxton et al., 2019); the involvement of some thermogenic activators in the control of blood pressure and endothelial function is also well documented: Prdm16 knocking out increases blood pressure (Becher et al., 2021), while endothelial-specific loss of function of peroxisome proliferator activated receptor gamma coactivator 1α, a transcriptional coactivator important for the Ucp1 expression, primes endothelial dysfunction leading to hypertension (Craige et al., 2016). The effects of *A. platensis* F&M-C256 on blood pressure may be due to vasoactive peptides produced by the digestion of the algal biomass. Interestingly, peptides purified from *Spirulina* reduced both systolic and diastolic blood pressure in spontaneously hypertensive rats by affecting the expression of local kidney Renin-Angiotensin System components, while other authors showed the same effects through a PI3K/AKT/eNOS-dependent mechanism (Zheng et al., 2017; Carrizzo et al., 2019).

5. Conclusions

In conclusion, our findings demonstrate that the beneficial effects of *A. platensis* F&M−C256 on cardiometabolic risk factors associated with MetS are at least in part due to the promotion of Ucp1-mediated browning of visceral fat and thermogenesis and to the reduction of ANGPTL3 and its inhibitory effect on LPL in adipose tissue (Fig. 7). Phycocyanin and PUFA, mainly γ-linolenic acid, can be responsible of these effects even if the contribution of other minor components such as

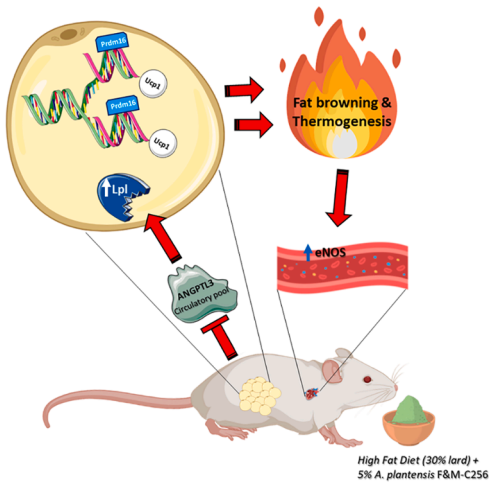

Fig. 7. Schematic summary of the mechanisms underlying the beneficial effects of *A. platensis* F&M-C256 on cardiometabolic risk factors associated with MetS. Prdm16: PR domain containing 16; Ucp1: uncoupling protein 1; LPL: lipoprotein lipase; eNOS: endothelial nitic oxide synthase. ANGPTL3: angiopoietin-like protein 3.

carotenoids cannot be excluded.

Although some exploratory clinical trials demonstrated the protective effects of *A. platensis* on metabolic abnormalities, the subgroup of patients that are most likely to benefit from this microalga, the optimal dose, period of administration and potential drug interactions, should be further investigated to verify the clinical usefulness of *A. platensis* supplementation as a new strategy for controlling MetS associated alterations.

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CRediT authorship contribution statement

Mario D'Ambrosio: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Elisabetta Bigagli:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Conceptualization. **Lorenzo Cinci:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Formal analysis. **Gianluca Cipriani:** Writing – review & editing, Resources, Methodology. **Alberto Niccolai:** Writing – review & editing, Investigation, Formal analysis. **Natascia Biondi:** Writing – review & editing, Resources, Methodology. **Liliana Rodolfi:** Writing – review & editing, Resources, Methodology. **Francesca Zambelli:** Writing – review & editing, Investigation, Formal analysis. **Manuela Gencarelli:** Writing – review & editing, Investigation, Formal analysis. **Annunziatina Laurino:** Writing – review & editing, Formal analysis. **Laura Raimondi:** Writing – review & editing, Resources, Methodology. **Mario R. Tredici:** Methodology, Funding acquisition. **Cristina Luceri:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106150.

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