RESEARCH PAPER

A mixture of four dietary fibres ameliorates adiposity and improves metabolic profile and intestinal health in cafeteria-fed obese rats: an integrative multi-omics approach

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Abstract

The aim of this study was to assess the effects of a mixture of four dietary fibers on obese rats. Four groups of male Wistar rats were fed with either standard chow (STD) or cafeteria diet (CAF) and were orally supplemented with either fibre mixture (2 g kg^{-1} of body weight) (STD+F or CAF+F groups) or vehicle (STD+VH or CAF+VH groups). We studied a wide number of biometric, biochemical, transcriptomic, metagenomic and metabolomic variables and applied an integrative multivariate approach based on multiple factor analysis and Pearson's correlation analysis. A significant reduction in body weight, adiposity, HbA1c and HDL-cholesterol serum levels, and colon MPO activity was observed, whereas cecal weight and small intestine length:weight ratio were significantly increased in F-treated groups compared to control animals. CAF+F rats displayed a significant enhancement in energy expenditure, fat oxidation and fresh stool weight, and a significant reduction in adiponectin and LPS serum levels, compared to control group. Animals in STD+F group showed reduced serum LDL-cholesterol levels and a significant reduction in total cholesterol levels in the liver compared to STF+VH group. The intervention effect was reflected at the metabolomic (i.e., production of short-chain fatty acids, phenolic acids, and amino acids), metagenomic (i.e., modulation of Ruminococcus and *Lactobacillus* genus) and transcriptomic (i.e., expression of tight junctions and proteolysis) levels. Altogether, our integrative multi-omics approach highlights the potential of supplementation with a mixture of fibers to ameliorate the impairments triggered by obesity in terms of adiposity, metabolic profile, and intestinal health.

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1. Introduction

Obesity is defined as an abnormal or excessive body fat accumulation that may impair health and is considered a major risk factor for many chronic diseases, including metabolic syndrome (MetS). MetS is a pathologic condition composed of a constellation of risk factors including central obesity, insulin resistance, systemic hypertension, and atherogenic dyslipidemia. These interrelated conditions can lead to a prothrombotic and pro-inflammatory state, commonly associated with accelerated atherosclerosis and increased risk of cardiovascular disease, among others. Among noncommunicable diseases, MetS and obesity are major health hazards worldwide and leading causes of mortality and morbidity [1,2].

Obesogenic high-calorie, low-fiber diets are considered one of the principal risk factors for obesity and MetS. Therefore, diet is among the most important modifiable behaviors for the prevention and amelioration of these multifactorial disorders. Indeed, dietary fiber intake is suggested to prevent diet-induced obesity and to lower the risk of MetS [3,4], having a direct impact on insulin resistance, glucose tolerance and weight control, while serving as a rich source of antioxidant and anti-inflammatory nutrients [5]. Moreover, fiber is well known to promote healthy gut microbiota composition, which can in turn modulate the host response to diet [3]. However, the available evidence suggests that dietary fiber consumption is associated only with small improvements in body weight, and the effects in terms of changes in body composition and fat mass are not conclusive [6]. Given their main properties (*i.e.*, viscosity, water solubility and fermentation rate), dietary fibers can be classified into different groups. For instance, inulin and resistant starch are defined as nonviscous, soluble, and fermentable fibers, whereas guar gum is a viscous, soluble and fermentable fiber. These properties determine the health effects of dietary fiber through its ability to be fermented by the intestinal microbiota and produce a prebiotic effect, to induce mechanical and laxative effects and, therefore, to regulate digestive function, and to reduce nutrient absorption in the intestine [7].

In such multifactorial disorders as obesity and MetS, it is fundamental to combine multidimensional data to get a comprehensive picture of the effects elicited by dietary interventions, describing intrinsic factors (*e.g.*, genetics, gut microbiome), extrinsic factors (*e.g.*, diet) and their interconnections. The combination of multiomics techniques, therefore, is a powerful strategy for broadening the current understanding of MetS alterations and the beneficial effects of these treatments.

The aim of this work was to evaluate the effect of an intervention with a fiber mixture comprising 36% inulin, 31.5% hydrolyzed guar gum, 22.5% resistant maltodextrin, and 10% dehydrated plum on an obese metabolic phenotype. To this end, rats with diet-induced obesity [8] were fed with a dose of fiber equivalent to human daily consumption [9]. The effects produced by the treatment were widely investigated, taking into consideration biometric, biochemical, transcriptomic, metagenomic, and metabolomic data, and using integrative multivariate statistical techniques.

2. Materials and methods

2.1. Animals, diets, and treatments

The Animal Ethics Committee of Rovira i Virgili University (Tarragona, Spain) and the *Generalitat de Catalunya* approved all the procedures (reference number 9823 of the Generalitat de Catalunya). The experimental protocol adhered to the "principles of laboratory animal care" and was carried out in accordance with the European Communities Council Directive (86/609/CEE).

The animals used in this study were 4-week-old male Wistar rats (Envigo, Barcelona, Spain) housed singly at 22 °C for a light/dark period of 12 h (lights on at 8 AM) and with free access to food and water. All animals were subjected to a 4-day adaptation period in which they voluntary consumed 200 μL of diluted low-fat condensed milk from a feeding syringe. After that, the rats were randomly distributed into four experimental groups (n=10) depending on the diet and the oral treatment received over 8 weeks: the STD+VH group was fed with a standard chow diet (STD, 2018 Teklad Global 18% Protein Rodent Diet, 3.1 kcal g^{-1}; Envigo, Barcelona, Spain) supplemented daily with 2 g kg^{-1} of body weight of maltodextrin dissolved in diluted low-fat condensed milk, which was diluted 1:4 with water (vehicle, VH); the STD+F group was fed with STD supplemented daily with 2 g kg^{-1} of body weight of a combination of fibers (F) dissolved in diluted low-fat condensed milk; the CAF+VH group was fed with an obesogenic cafeteria diet (CAF) supplemented daily with 2 g kg^{-1} of body weight of maltodextrin dissolved in diluted low-fat condensed milk; and the CAF+F group was fed with CAF supplemented daily with 2 g kg^{-1} of body weight of the F dissolved in diluted low-fat condensed milk. The CAF diet was prepared as previously described [8]. The F treatment consisted of a mixture of four different natural components, comprising 36% inulin, 31.5% hydrolyzed guar gum, 22.5% resistant maltodextrin, and 10% dehydrated plum (Laboratorios Ordesa S.L., Barcelona, Spain). This treatment contained a total of 78% of fibers, of which 75% were fermentable, while the remaining 22% was mainly composed of other carbohydrates (*e.g.*, glucose, sucrose). Given an average rat's weight of 270 g, the dose of 2 g kg^{-1} day^{-1} of the F is equivalent to the daily consumption of 22.4 g of fiber by a 60 kg human [9]. In most countries, the recommended daily intake of dietary fiber is 25–35 g [10], so the dose of F used in this study can be considered acceptable for human consumption.

The body weight and food intake data were recorded once a week. In the seventh week of the study, the animals were housed in metabolic cages (3700M071 model for rats from 150 g to 300 g, Panlab, Barcelona, Spain) to collect urine for a period of 24-h in fasting conditions. Urine samples were stored at -80 °C in tubes containing 270 mg of boric acid. In the eighth week of the study, rats were fasted for seven hours and were subjected to an oral glucose tolerance test (OGTT) following a previously described procedure [11].

After eight weeks, the animals were deprived of food for 6 hours (from 8 h to 14 h), anaesthetized with sodium pentobarbital (Merck Life Science, Madrid, Spain), and blood was collected through cardiac puncture. Serum and plasma were obtained from blood samples by centrifugation and were stored at -80°C until further analysis. PBMCs were obtained as previously described [12] and PBMC pellets were stored at -80 °C until obtaining RNA. White adipose tissue depots (retroperitoneal-RWAT, inguinal-IWAT, mesenteric-MWAT and epididymal-EWAT), the liver, small intestine, colon, and cecum (with the contents) were rapidly removed after death, weighed, frozen in liquid nitrogen and stored at -80 °C until further analysis. The length of the small intestine and colon were recorded as measures related to inflammation.

2.2. Serum, plasma, and tissue biochemical analysis

Biochemical analyses of serum, liver, and colon samples were performed using the methodology described in the Supplementary Material file. The homeostasis model assessment-estimated insulin resistance (HOMA-IR) index was calculated following the formula proposed by Matthews [13]. Additionally, the insulin sensitivity was assessed by the revised quantitative insulin sensitivity

check index (R-QUICKI), using the formula described by Perseghin [14].

2.3. Adiposity

The adiposity level of each animal was determined via an adiposity index computed as the sum of the RWAT, IWAT, MWAT, and EWAT depot weights or, in the case of the visceral adiposity index, the sum of the RWAT, IWAT, and EWAT depot weights, and expressed as a percentage of total body weight.

2.4. Body composition analysis

Lean, fat and total water measurements (in percentage of body weight) were performed three times for each animal during the study – at baseline, at 4 weeks and before sacrifice – by quantitative magnetic resonance using an EchoMRI-700 device (Echo Medical Systems, L.L.C., Houston, TX, USA) [11,13,14].

2.5. Indirect calorimetry and locomotor activity

The OxyletPro system (Panlab, Barcelona, Spain) was used to evaluate the animals' physical activity and respiratory metabolism. The measurements were performed during weeks five and six of the study for 23 h (from 9 h to 8 h of the following day). Data collected during the first 4 h were discarded, established as an acclimatation period, which finally corresponded to a record of 19 h per animal. During the first few hours (from 12 h to 17 h), the animals were left with water but without food to obtain the basal metabolism values (fasting; basal oxidation of carbohydrates and lipids). At 17 h, each animal was fed with the corresponding diets (STD or CAF) to obtain the values under *ad libitum* conditions. This procedure has been described by other authors previously [15]. Locomotor activity was also measured as previously described [8].

2.6. Fecal analysis

During the last week of the study, the frequency, weight and water content of the animals' feces were measured over a 24-h period. During the first 2 h, fresh feces were collected and quantified for consistency and water content measurements. The qualitative appearance of the fresh feces was evaluated using a three-point scale: normal, semi-solid, and diarrhea [16]. Water content was determined as the weight difference between fresh and dry stools obtained after a 24-h period at 70 °C, with the result being expressed as the percentage of fresh feces.

The same 24-h fecal samples were used for lipid absorption analysis. Fecal lipid content was analyzed using a gravimetry protocol described previously [17]. Lipid absorption was calculated from the total lipid content values in feces normalized for animals' lipid consumption with the results expressed as percentages.

2.7. Multi-omics analysis of serum, urine, cecum, and PBMC samples

Quantitative large-scale metabolomics analyses of serum and urine samples were conducted following the methodology described elsewhere with slight modifications [18]. ^{1}HNMR metabolic profiling analysis was performed following the procedure described by Palacios-Jordan et al. [19]. Detailed information about the metabolomic, transcriptomic and metagenomic methodologies employed in this study can be found in the Supplementary Material file.

2.8. Statistical analysis

All the data showed throughout the work are expressed as mean $\pm$ SEM. The Grubbs test and principal component analysis (PCA) were used to detect outliers, which were discarded for subsequent analyses. The homoscedasticity among groups was assessed using Levene's test, and when this condition was not accomplished, data were transformed to a base-10 logarithm to obtain similar variances before statistical testing. Variables within each data set (*i.e.*, biometrical/biochemical variables, NMR serum metabolomics, LC/MS serum metabolomics, LC/MS urine metabolomics, metagenomics) were compared among the experimental groups by using two-way analysis of variance (ANOVA), considering the diet (D: STD or CAF), the treatment (T: VH or F) and their interaction (D $\times$ T). For significant D $\times$ T interactions, Fisher's LSD post hoc and Student's t-tests were performed two-to-two among the different groups. The temporal evolution of some of the variables were analyzed by repeated-measures (RMs) ANOVA, with time as a within-subject factor and diet or treatment as a between-subject factor. All these statistical analyses were performed using the statistical software package SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). *P*-values <.05 were considered statistically significant.

For transcriptomic data analysis, sparse partial least squares discriminant analysis (sPLS-DA) using mixOmics package [20] was employed to select those genes with the greatest differences in expression between the two groups analyzed and with the greatest importance in component 1. The GeneSCF program (http://genescf.kandurilab.org/documentation.php) was then used to analyze which processes or metabolic pathways were most represented by the genes that showed the greatest differences in expression between groups, employing Fisher's exact test with various corrections for multiple comparisons. From the list of 200 selected genes, only those with a gene name or EntrezGeneID code were finally selected. The EntrezGeneID was used to search the *Rattus norvegicus* KEGG metabolic database (rno code in the KEGG database). To calculate the statistical parameters, 20,000 background genes were used.

Afterwards, multiple factor analysis (MFA) was performed for integrating all data sets with the aim of exploring the contribution of the different variables to the effects provoked by the treatment. MFA was performed by using only significant variables according to two-way ANOVA results for removing non-informative variables to sharpen the separation between the study groups. The MFA models were graphically displayed by plotting the projections of the variables (biometrical and biochemical; UHPLC-MS/MS metabolomics on serum and urine; NMR metabolomics on serum; metagenomics on family, genus and phylum levels; and transcriptomics) and samples (*i.e.*, group label) onto the bidimensional space defined by the first two dimensions. Finally, Pearson correlations were computed on significant variables (*P*-values <.05) to search for associations between metabolomics, metagenomics and biometrical/biochemical variables. Considering the differential impact of F treatment in STD- and CAF-fed animals, correlation analyses were conducted separately in each of the two diet groups. Correlations with *P*-values ≤.01 were considered significant and were graphically represented. All the data were normalized by means of log transformation and Pareto scaling prior to carrying out these statistical analyses using the R software packages FactoMineR and Factoextra (http://www.r-project.org).

3. Results and discussion

In this study, we evaluated the effect of a mixture of fibers with a high content of fermentable fibers (F), using a daily dose extrap-
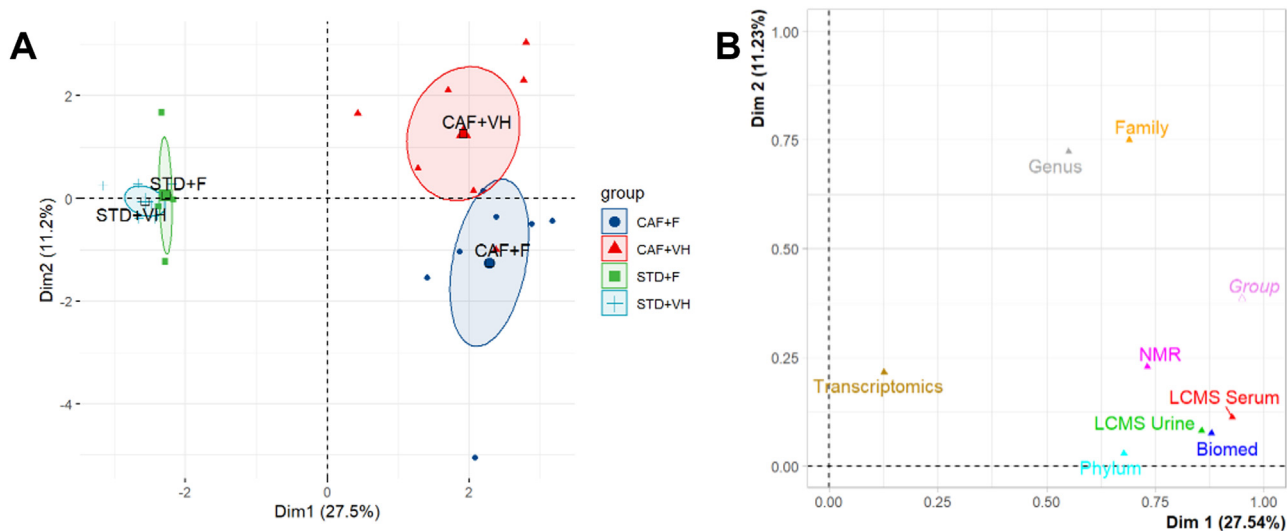
Fig. 1. Summary of multiple factor analysis (MFA) results. Scatter plots showing the projection of individual animals from the four study groups (*i.e.*, STD+VH, STD+F, CAF+VH, CAF+F) in the space defined by the first two dimensions, using the significant variables according to two-way ANOVA (A). Variable representation plots showing the projection of the different data sets (*i.e.*, biometrical/biochemical, NMR serum metabolomics, LC/MS serum metabolomics, LC/MS urine metabolomics, transcriptomics, metagenomics) in the space defined by the first two dimensions, using the significant variables according to two-way ANOVA (B).

olated to humans, on obesity and related disorders. Furthermore, we investigated the mechanisms and metabolic pathways potentially involved in the effects elicited by this intervention by means of the measurement of a wide number of biometric and biochemical parameters, the use of a multi-omics approach and their integration through multivariate analysis.

3.1. Multi-omics data integration

MFA modelling provided clear differentiation between CAF and STD groups along the first dimension, whereas F supplementation induced the separation of F-fed and VH-fed animals along the second dimension mainly in the CAF group (Fig. 1A). Overall, this is in line with the more pronounced response to the F treatment observed in CAF-fed animals, which could be understood as an adaptative mechanism to counteract the alteration of energy homeostasis produced by CAF consumption [8,17,21]. The variable representation plot (Fig. 1B) evidenced that biometrical and biochemical variables together with metabolomic data were the major drivers in the multivariate differentiation of the four study groups. Metagenomic variables also had a significant impact on the two first MFA dimensions, whereas the contribution of transcriptomic data was minor. Afterwards, Pearson's correlation analyses were conducted to investigate the association among the most significant variables according to two-way ANOVA and MFA results (Figs. 2 and 3). Altogether, this integrative multi-omics analysis enabled us to comprehensively elucidate the molecular mechanisms and biological pathways influenced by the F intervention, as detailed in the following sections.

3.2. F supplementation promotes a healthier body composition and metabolic profile

The results showed a clear reduction of body weight as a result of F treatment, with decreases of 11% and 6% in body weight gain in the STD+F and CAF+F groups, respectively (Table 1). In addition, a reduction in relative and absolute weights of EWAT depot and in absolute weight of MWAT depot was observed (Table 1), without affecting the energy intake of the animals (Supplementary Fig. 1).

Furthermore, although no significant differences were observed regarding the percentage of fat mass, lean mass, lean mass:fat mass ratio or body water content during the intervention in either dietary group (Fig. 4A, 4C–E), F treatment significantly reduced the fat mass of the animals at the end of the study, with decreases of 6% and 16% in the STD+F and CAF+F groups, respectively (Fig 4B). In fact, F treatment significantly decreased the adiposity index and showed a clear tendency (two-way ANOVA, $P=.05$ for T) to reduce the visceral adipose index of the animals (Table 1). These results are relevant since excessive visceral adipose tissue accumulation has been shown to be closely related to the appearance of cardiovascular diseases, type 2 diabetes and increased mortality risk [22]. Interestingly, the body weight gain of the animals was positively correlated with MWAT depot, adiposity index and body fat mass (Fig 2), reinforcing the hypothesis that F treatment reduces body weight through a decrease of body fat in animals. This F-mediated enhancement of the body composition profile, without a significant reduction in energy intake, has been previously described by different authors in both animal and human studies [7,23]. Moreover, our results concur with several studies suggesting that fermentable fiber consumption produces a change in body fat distribution towards a healthier profile [7].

Leptin and adiponectin are considered plasma biomarkers of adipose tissue functionality, which are produced at levels proportional to the body fat content [21]. In this sense, agreeing with the observed F effect on fat reduction in CAF-fed animals, F treatment produced a global tendency to decrease the circulating levels of leptin (two-way ANOVA, $P=.08$ for T) and a significant decrease of adiponectin serum levels in CAF-fed animals (Student's t-test, $P<.05$) compared to animals treated with vehicle (Table 1). Contradictory results can be found in the literature in this regard, since various authors have reported that soluble fiber consumption is associated with an increase in adiponectin levels [24], whereas other studies did not detect any effect of fermentable fiber on this biomarker [25]. It is important to highlight that serum adiponectin levels relative to the adipose tissue mass did not differ as a result of the intervention (Table 1), thereby indicating an effective production of adiponectin by adipose tissue [26].

In transcriptomics analysis, significant differences were observed in several metabolic pathways in CAF fed animals, but not
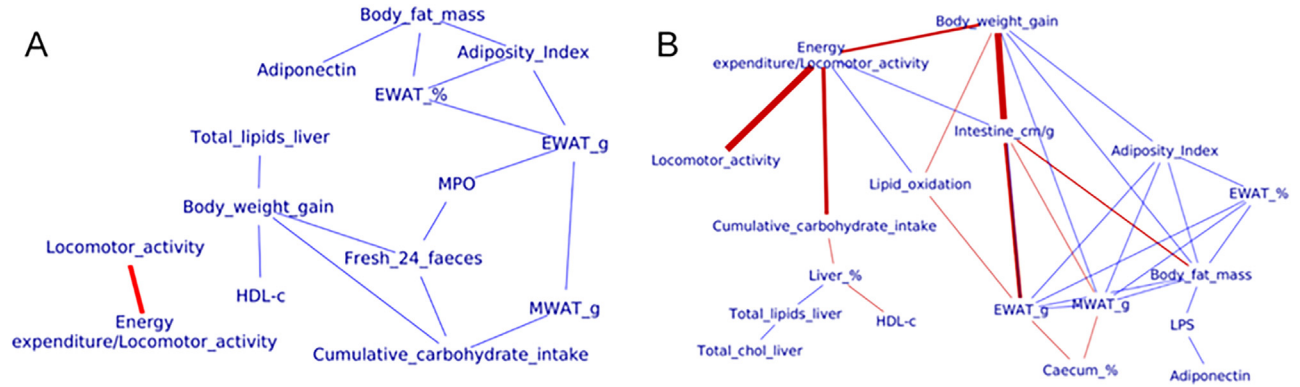
Fig. 2. Network representation of the associations between biometrical/biochemical parameters as a result of F treatment in animals fed the STD diet (A) and the CAF diet (B). Pearson correlations were computed on significant variables and significant correlations (P-value ≤.01) were represented. Positive correlations are shown in blue and negative correlations in red. The thickness of the line refers to the value of the correlation, the correlation being greater the thicker the line.

Table 1
Biometric and serum concentration parameters in rats fed a standard diet (STD) or cafeteria diet (CAF) and treated with vehicle (VH) or fiber (F)

|	STD+VH	STD+F	CAF+VH	CAF+F	ANOVA
Initial body weight (g)	97.7 ± 2.30	99.0 ± 1.70	95.3 ± 1.70	96.5 ± 1.80	-
Final body weight (g)	374 ± 11	344 ± 6	447 ± 17	426 ± 14	D
Δ Body weight (g)	277 ± 11	245 ± 5	352 ± 16	330 ± 13	D, T
RWAT (g)	8.02 ± 0.51	7.08 ± 0.52	19.4 ± 1.30	17.1 ± 1.30	D
RWAT (%)	2.93 ± 0.21	2.80 ± 0.19	5.48 ± 0.19	5.12 ± 0.22	D
IWAT (g)	4.35 ± 0.32	3.91 ± 0.17	10.4 ± 1.10	8.2 ± 0.60	D
IWAT (%)	1.57 ± 0.09	1.55 ± 0.09	2.90 ± 0.17	2.50 ± 0.15	D
MWAT (g)	5.24 ± 0.21	4.43 ± 0.20	10.7 ± 0.80	9.3 ± 0.80	D, T
MWAT (%)	1.92 ± 0.10	1.76 ± 0.07	3.02 ± 0.13	2.80 ± 0.14	D
EWAT (g)	7.57 ± 0.41	5.75 ± 0.37	17.8 ± 1.40	14.8 ± 1.40	D, T
EWAT (%)	2.78 ± 0.18	2.29 ± 0.16	5.00 ± 0.26	4.41 ± 0.30	D, T
Adiposity index	9.19 ± 0.49	8.54 ± 0.40	16.4 ± 0.60	14.8 ± 0.70	D, T
Visceral adiposity index	7.63 ± 0.46	6.85 ± 0.35	13.5 ± 0.50	12.3 ± 0.60	D
Leptin (ng/mL)	9.23 ± 0.94	8.70 ± 0.91	47.21 ± 5.76	31.98 ± 2.98	D
Adiponectin (μg/mL)	21.95 ± 1.56	22.49 ± 2.66	34.96 ± 2.59	24.55 ± 1.36	D, T, DxT
Leptin:Adiponectin Ratio	0.40 ± 0.06	0.36 ± 0.04	1.36 ± 0.15	1.34 ± 0.14	D
Adiponectin:Fat mass Ratio	0.62 ± 0.99	0.73 ± 0,20	0.35 ± 0.09	0.31 ± 0.09	D

Wistar rats supplemented with fiber (F) or vehicle (VH) and fed a cafeteria (CAF) diet or standard (STD) diet for 8 weeks. EWAT, epididymal white adipose tissue; MWAT, mesenteric white adipose tissue; IWAT, inguinal white adipose tissue; and RWAT, retroperitoneal white adipose tissue is expressed as absolute weight (g) and relative weight (%), calculated according to the formula (100*tissue weight/body weight). The adiposity index and visceral adipose index were computed as the sum of EWAT, MWAT, IWAT and RWAT depot weights (in grams) or the sum of RWAT, IWAT and EWAT depot weights (in grams), respectively, and expressed as a percentage of body weight. Data are given as mean ± SEM. D, diet effect; T, fiber treatment effect; DxT, diet-treatment interaction (Two-way ANOVA test, $P<.05$).

when comparing STD+VH and STD+F groups (*data not shown*). In line with the results discussed above on the beneficial effect of F on body weight and body fat distribution, F treatment produced a significant change in the thermogenic pathway by decreasing the expression levels of several related genes in PBMCs, such as *NADH: ubiquinone oxidoreductase subunit C2, protein kinase cAMP-activated catalytic subunit beta and ATP synthase* (Table S1). In this regard, previous studies with animals have described that the consumption of high-fat diets, including the CAF diet, increases the thermogenic capacity in an attempt to dissipate excessive energy intake and maintain body weight energy [28].

3.3. The administration of F decreases locomotor activity and enhances energy expenditure and lipid oxidation in CAF-fed rats

F treatment produced a significant reduction in global activity (Fig. 5A), which could suggest a treatment effect on anxiety and stress, as reported in other studies [27]. No significant changes

were observed in energy expenditure analysis (Fig. 5B). However, when corrected by animal activity, a significant energy expenditure enhancement was found in CAF-fed animals treated with F (Student's t-test, $P<.05$) (Fig. 5C). In addition, an increase in fat oxidation in the feeding period was observed in the CAF+F group (Student's t-test, $P<.05$) (Fig. 5D), but no significant effects were observed on carbohydrate oxidation (Fig. 5E) either in the CAF-fed or STD-fed groups. These results suggest that the increased energy expenditure seems to be due to enhanced lipid oxidation, a finding that was corroborated in correlation analysis, since lipid oxidation was positively correlated with energy expenditure and negatively correlated with the body weight of the animals fed with the CAF diet (Fig. 2B). Furthermore, in CAF-fed animals, carbohydrate intake negatively correlated with energy expenditure corrected by locomotor activity (Fig. 2B). In these animals, F treatment showed a tendency to decrease the preference for carbohydrate consumption (Student's t-test, $P=.090$) (Fig. 5E), which could be explained by the noticed less intake of sweetened milk (289±12 mL day^{-1}
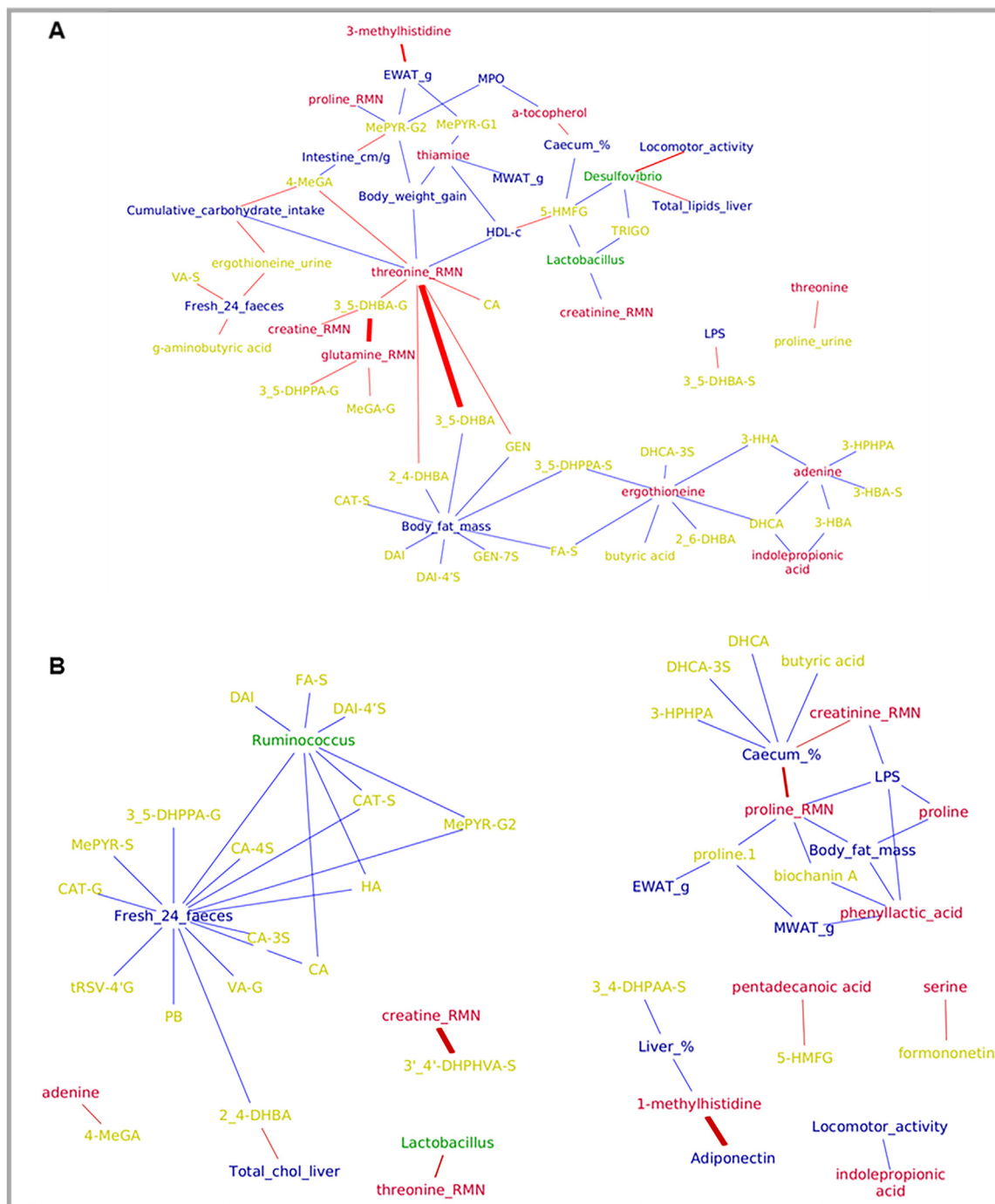
Fig. 3. Network representation of the associations between metabolomic, metagenomic and biometrical/biochemical variables as a result of F treatment in animals fed the STD diet (A) and the CAF diet (B). Pearson correlations were computed on significant variables and significant correlations (*P*-value ≤.01) were represented. Positive correlations are shown in blue and negative correlations in red. The thickness of the line refers to the value of the correlation, the correlation being greater the thicker the line. Label colors differentiate the different sets of measures: blue for biometrical/biochemical parameters; red for serum metabolomics; yellow for urine metabolomics; green for metagenomics.

in the CAF+VH group *vs.* 242±13 mL day^{-1} in the CAF+F group, Student's t-test, *P*=.016), the most consumed food in the CAF diet-fed animals. According to these results [28], F addition significantly reduced the circulating levels of pentadecanoic acid in CAF fed animals (Table 2), a marker of the intake of dairy products [28]. These results suggest that the reduction in carbohydrate intake could mediate, along with the increase in lipid oxidation, the beneficial effects of F on body weight and adiposity reduction in CAF diet-fed animals treated with F.

3.4. F supplementation improves cholesterol metabolism and HbA1c blood levels

No significant effects of F treatment were observed in CAF-fed animals on total cholesterol, LDL cholesterol, triglyceride, or NE-FAs blood levels, nor on lipid absorption. However, in STD-fed animals, F treatment produced a tendency towards a reduction in LDL cholesterol serum levels (Student's t-test, *P*<.1) and significantly decreased total cholesterol levels in the liver (Student's t-

Table 2
Metabolomic alterations induced in serum and urine by the dietary intervention with fibres in rats fed standard diet (STD) or cafeteria diet (CAF). Metabolite concentrations are expressed as mean $\pm$ standard deviation (μg L^{-1} for UHPLC-MS/MS measurements, nmol L^{-1} for ^{1}H-NMR measurements)

Metabolite	STD+VH	STD+F	CAF+VH	CAF+F	ANOVA
UHPLC-MS/MS serum					
amino acids and derivatives					
serine	485.0 $\pm$ 187.6	627.0 $\pm$ 96.2	941.5 $\pm$ 442.4	638.7 $\pm$ 116.2	T, DxT
threonine	2932.5 $\pm$ 791.2	3655.5 $\pm$ 244.8	5002.6 $\pm$ 1371.0	3970.9 $\pm$ 1276.0	T, DxT
proline	11064.0 $\pm$ 2556.5	11657.2 $\pm$ 1204.9	14848.2 $\pm$ 3443.5	10278.9 $\pm$ 2036.1	T, DxT
1-methylhistidine	311.8 $\pm$ 64.3	288.4 $\pm$ 90.8	356.4 $\pm$ 89.0	535.8 $\pm$ 168.4	D, T, DxT
3-methylhistidine	1721.1 $\pm$ 516.8	2076.3 $\pm$ 372.7	1909.6 $\pm$ 748.4	2744.9 $\pm$ 940.6	T
ergothioneine	587.1 $\pm$ 150.1	644.5 $\pm$ 121.9	261.9 $\pm$ 51.6	202.6 $\pm$ 20.8	D, T, DxT
microbiota metabolites - aromatic amino acids					
phenyllactic acid	24.3 $\pm$ 14.3	28.2 $\pm$ 14.1	53.2 $\pm$ 45.3	23.8 $\pm$ 13.7	T, DxT
phenylacetylglycine	191.9 $\pm$ 154.1	217.0 $\pm$ 109.6	140.5 $\pm$ 78.5	74.3 $\pm$ 31.3	D, T, DxT
phenylacetylglutamine	5.9 $\pm$ 6.2	8.7 $\pm$ 6.2	14.8 $\pm$ 19.9	3.5 $\pm$ 2.4	T, DxT
indolepropionic acid	785.3 $\pm$ 228.4	668.0 $\pm$ 229.2	924.1 $\pm$ 410.6	618.7 $\pm$ 575.0	T
vitamins					
thiamine	1387.6 $\pm$ 486.5	925.0 $\pm$ 280.3	762.8 $\pm$ 134.1	935.1 $\pm$ 285.4	DxT
riboflavin	81.3 $\pm$ 23.3	68.8 $\pm$ 13.2	62.3 $\pm$ 8.7	52.2 $\pm$ 6.6	D, T
α-tocopherol	9620.3 $\pm$ 2186.9	6559.9 $\pm$ 2583.2	5907.9 $\pm$ 1552.7	6507.9 $\pm$ 910.7	DxT
free fatty acids					
pentadecanoic acid	ND	ND	8232.5 $\pm$ 3510.4	3528.4 $\pm$ 2959.3	D, T, DxT
amines					
adenine	16.1 $\pm$ 4.8	15.0 $\pm$ 2.5	16.4 $\pm$ 3.9	12.3 $\pm$ 2.1	T
^{1}H-NMR serum					
Creatine	0.1 $\pm$ 0.004	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01	0.16 $\pm$ 0.01	D, T
Creatinine	9.25 $\pm$ 0.45	10.4 $\pm$ 0.30	10.9 $\pm$ 0.72	9.63 $\pm$ 0.60	DxT
Phenylalanine	44.7 $\pm$ 1.38	45.4 $\pm$ 1.35	48.8 $\pm$ 1.35	42.0 $\pm$ 0.799	D, T, DxT
Proline	0.094 $\pm$ 0.01	0.087 $\pm$ 0.01	0.112 $\pm$ 0.01	0.088 $\pm$ 0.004	T
Glutamine	0.28 $\pm$ 0.01	0.31 $\pm$ 0.01	0.32 $\pm$ 0.01	0.27 $\pm$ 0.01	DxT
Threonine	0.12 $\pm$ 0.01	0.13 $\pm$ 0.004	0.18 $\pm$ 0.01	0.15 $\pm$ 0.003	D, T, DxT
UHPLC-MS/MS urine					
amino acids and derivatives					
proline	977.4 $\pm$ 304.9	791.2 $\pm$ 267.5	1212.0 $\pm$ 689.1	808.4 $\pm$ 311.8	T
ergothioneine	514.7 $\pm$ 94.9	543.0 $\pm$ 66.3	371.2 $\pm$ 46.7	426.9 $\pm$ 57.6	D, T
microbiota metabolites - aromatic amino acids					
phenylacetylglycine	68959.4 $\pm$ 15461.7	52043.1 $\pm$ 11411.9	40407.6 $\pm$ 12765.8	31564.8 $\pm$ 16129.9	D, T
microbiota metabolites - phenolic acids					
3-hydroxybenzoic acid (3-HBA)	54.1 $\pm$ 48.9	47.0 $\pm$ 45.8	35.8 $\pm$ 13.3	75.8 $\pm$ 40.9	D, T, DxT
3-hydroxybenzoic acid sulfate (3-HBA-S)	8.8 $\pm$ 7.2	9.5 $\pm$ 9.8	5.8 $\pm$ 2.6	23.0 $\pm$ 27.1	D, T, DxT
2,4-dihydroxibenzoic acid (2,4-DHBA)	23.2 $\pm$ 8.8	18.0 $\pm$ 10.0	4.8 $\pm$ 2.1	6.9 $\pm$ 2.6	D, T, DxT
2,6-dihydroxibenzoic acid (2,6-DHBA)	277.9 $\pm$ 96.4	226.9 $\pm$ 68.9	78.2 $\pm$ 54.5	105.3 $\pm$ 43.6	D, T, DxT
3,4-dihydroxibenzoic acid (3,4-DHBA)	557.7 $\pm$ 1167.1	345.9 $\pm$ 339.5	349.8 $\pm$ 205.0	719.9 $\pm$ 322.0	D, T
3,5-dihydroxibenzoic acid (3,5-DHBA)	581.7 $\pm$ 512.2	321.4 $\pm$ 171.4	116.2 $\pm$ 52.9	251.0 $\pm$ 177.8	T, DxT
3,5-dihydroxibenzoic acid glucuronide (3,5-DHBA-G)	19.6 $\pm$ 11.3	11.1 $\pm$ 6.0	5.3 $\pm$ 3.8	20.8 $\pm$ 23.7	T, DxT
3,5-dihydroxibenzoic acid sulfate (3,5-DHBA-S)	44.8 $\pm$ 13.8	34.4 $\pm$ 15.8	18.9 $\pm$ 14.0	68.1 $\pm$ 99.5	T, DxT
hippuric acid (HA)	206091.4 $\pm$ 121497.1	204201.2 $\pm$ 57038.3	74803.0 $\pm$ 36328.0	123096.7 $\pm$ 37038.3	D, T
3-hydroxyhippuric acid (3-HHA)	284.2 $\pm$ 327.9	255.6 $\pm$ 278.3	108.8 $\pm$ 50.4	226.1 $\pm$ 106.1	T
vanillic acid glucuronide (VA-G)	96.4 $\pm$ 34.2	80.5 $\pm$ 30.8	77.2 $\pm$ 36.1	113.9 $\pm$ 51.3	D, T, DxT
vanillic acid sulfate (VA-S)	270.8 $\pm$ 63.3	290.2 $\pm$ 95.7	394.2 $\pm$ 95.1	765.1 $\pm$ 712.4	D, T, DxT
4-methylgallic acid (4-MeGA)	6.9 $\pm$ 4.4	8.9 $\pm$ 4.1	9.9 $\pm$ 4.7	21.2 $\pm$ 10.2	D, T
3-methylgallic acid (3-MeGA)	7.9 $\pm$ 5.9	5.5 $\pm$ 2.4	5.7 $\pm$ 3.2	8.2 $\pm$ 3.5	D, T, DxT
methylgallic acid glucuronide (MeGA-G)	5.1 $\pm$ 1.3	4.5 $\pm$ 1.8	3.2 $\pm$ 2.7	5.2 $\pm$ 2.9	T, DxT
3,4-dihydroxyphenylacetic acid sulfate (3,4-DHPAA-S)	60641.8 $\pm$ 22766.5	58082.5 $\pm$ 12291.9	38190.4 $\pm$ 6702.8	50329.4 $\pm$ 10990.4	T, DxT
p-coumaric acid glucuronide (pCOU-G)	21.0 $\pm$ 15.0	21.0 $\pm$ 27.0	2.6 $\pm$ 5.2	7.5 $\pm$ 7.0	DxT
m-coumaric acid sulfate (mCOU-S)	13215.6 $\pm$ 9417.5	13538.9 $\pm$ 11199.4	2705.3 $\pm$ 1444.6	5234.4 $\pm$ 3643.7	D, T
caffeic acid (CA)	64.1 $\pm$ 31.3	59.6 $\pm$ 34.4	27.7 $\pm$ 19.0	60.8 $\pm$ 22.6	T, DxT
caffeic acid 3-sulfate (CA-3S)	20.8 $\pm$ 20.2	19.2 $\pm$ 21.5	5.3 $\pm$ 4.9	14.0 $\pm$ 14.5	T, DxT
caffeic acid 4-sulfate (CA-4S)	32.6 $\pm$ 7.9	35.2 $\pm$ 12.9	18.5 $\pm$ 10.7	46.9 $\pm$ 49.2	T, DxT
ferulic acid glucuronide (FA-G)	875.8 $\pm$ 825.1	1103.7 $\pm$ 1515.7	63.6 $\pm$ 172.9	104.1 $\pm$ 131.6	D, T, DxT
ferulic acid sulfate (FA-S)	7252.5 $\pm$ 4845.7	4961.5 $\pm$ 2607.7	2058.9 $\pm$ 1412.3	2665.6 $\pm$ 1221.0	D, T, DxT
3,5-dihydroxyphenylpropionic acid glucuronide (3,5-DHPPA-G)	3393.5 $\pm$ 614.0	3028.7 $\pm$ 1162.4	1982.7 $\pm$ 1709.5	3237.2 $\pm$ 1851.9	T, DxT
3,5-dihydroxyphenylpropionic acid sulfate (3,5-DHPPA-S)	19803.8 $\pm$ 12391.2	17193.2 $\pm$ 6440.8	3424.0 $\pm$ 2187.4	5088.9 $\pm$ 1932.2	D, T, DxT
dihydrocaffeic acid (DHCA)	105.5 $\pm$ 82.0	95.3 $\pm$ 55.1	73.0 $\pm$ 23.8	132.1 $\pm$ 67.3	T, DxT
dihydrocaffeic acid 3-sulfate (DHCA-3S)	569.5 $\pm$ 242.5	519.0 $\pm$ 219.0	173.4 $\pm$ 68.2	319.1 $\pm$ 200.6	D, T, DxT

Table 2 (*continued*)

Metabolite	STD+VH	STD+F	CAF+VH	CAF+F	ANOVA
dihydroferulic acid sulfate (DHFA-S)	1660.0 ± 1739.6	833.7 ± 307.2	531.8 ± 429.1	760.3 ± 475.9	DxT
3-(hydroxyphenyl)-hydroxypropionic acid (3-HPHPA)	13.8 ± 19.6	11.5 ± 17.3	6.2 ± 3.9	18.4 ± 12.9	D, T, DxT
methylpyrogallol glucuronide (isomer 1) (MePYR-G1)	27.0 ± 5.9	18.8 ± 7.8	30.8 ± 22.0	45.2 ± 25.0	D, T, DxT
methylpyrogallol glucuronide (isomer 2) (MePYR-G2)	193.1 ± 75.0	109.0 ± 63.4	124.9 ± 118.9	281.2 ± 301.0	DxT
methylpyrogallol sulfate (isomer 2) (MePYR-S2)	79.4 ± 83.4	42.5 ± 39.9	53.9 ± 29.1	145.2 ± 154.1	D, T, DxT
catechol glucuronide (CAT-G)	283.6 ± 360.3	372.5 ± 655.6	336.9 ± 333.1	1015.0 ± 1028.6	D, T, DxT
catechol sulfate (CAT-S)	775228.5 ± 292572.3	702821.1 ± 147289.5	323083.2 ± 112463.6	438712.1 ± 137717.0	D, T, DxT
short chain fatty acids					
butyric acid	3980.2 ± 1442.7	4009.1 ± 1166.1	1842.5 ± 805.6	2590.6 ± 1013.5	D, T
γ-aminobutyric acid	268.7 ± 75.9	286.3 ± 93.3	205.7 ± 89.6	288.5 ± 74.5	T
food-related metabolites – hydroxyphenyl-valerolactones					
4-hydroxy-5-(3′,4′-dihydroxyphenyl)-valeric acid sulfate (3,4-DHPVA-S)	3.6 ± 6.4	5.3 ± 7.5	14.1 ± 9.5	6.4 ± 9.4	T, DxT
5-(3′,4′-dihydroxyphenyl)-γ-valerolactone 3′-glucuronide (3,4-DHPV-3G)	ND	0.5 ± 0.9	1.8 ± 0.8	1.4 ± 1.0	D, DxT
food-related metabolites - isoflavones					
daidzein (DAI)	1058.6 ± 505.1	706.9 ± 229.8	271.1 ± 189.8	421.5 ± 181.6	D, T, DxT
daidzein 4′-sulfate (DAI-4′S)	2504.6 ± 1692.9	1451.2 ± 648.5	737.5 ± 564.2	1200.4 ± 548.0	T, DxT
genistein (GEN)	830.5 ± 508.1	492.5 ± 210.8	256.3 ± 204.1	345.3 ± 175.9	T, DxT
genistein 7-sulfate (GEN-7S)	97.4 ± 137.6	29.4 ± 35.6	29.7 ± 34.6	38.6 ± 32.7	DxT
biochanin A (BioA)	16.7 ± 15.2	6.0 ± 3.7	5.5 ± 6.0	6.9 ± 4.2	DxT
formononetin (FOR)	3.3 ± 1.4	2.5 ± 1.4	1.5 ± 1.0	2.3 ± 0.8	T, DxT
food-related metabolites - flavones					
luteolin (LUT)	1.4 ± 0.7	0.7 ± 0.5	0.6 ± 0.4	1.2 ± 1.1	T, DxT
food-related metabolites - stilbenoids					
trans-resveratrol 4′-glucuronide (tRSV-4′G)	16.4 ± 9.8	7.2 ± 4.7	3.0 ± 3.0	4.9 ± 2.9	DxT
food-related metabolites - betaines					
proline betaine	3579.8 ± 857.1	3633.1 ± 938.7	812.7 ± 481.7	1159.3 ± 468.7	D, T
trigonelline	32874.2 ± 9812.2	39288.0 ± 18354.8	551.2 ± 1743.2	2803.9 ± 3592.7	D, T, DxT
food-related metabolites - others					
5-(hydroxymethyl-2-furoyl)glycine (5-HMFG)	136.7 ± 35.8	1445.4 ± 557.0	155.5 ± 64.2	1715.5 ± 716.1	T

test, $P<.05$) (Table 3). The treatment with F did not produce any effect either on total liver lipids or on triglyceride levels either in STD-fed groups or in CAF-fed groups (Table 2,3). These results are in line with other studies demonstrating that fiber consumption produces a reduction in LDL cholesterol without affecting triglyceride levels [7]. This effect could be associated with the fermentation of soluble fiber by the intestinal microbiota and the production of short-chain fatty acids (SCFAs), which inhibit liver cholesterol synthesis [29], although in our study no correlations were observed between serum or liver LDL levels and SCFAs in STD-fed animals (Fig. 2A). It should be also mentioned that the CAF+F group showed a significant increase in liver relative weight compared to the CAF+VH group (Student's t-test, $P<.05$), while no effects were observed between STD-fed groups. This could be due to the observed reduction of body weight in F-treated groups or to factors other than the fat content in this organ, such as increased blood supply. In contrast, our results were not consistent with different studies describing that fiber consumption produces either no effect or an increase in plasma HDL cholesterol levels [7]. The reduction of HDL cholesterol by F treatment would be a non-beneficial effect (Table 3), since this cholesterol fraction is related to lower risk of cardiovascular disease development by decreasing the risk of atherosclerosis [30]. However, it should be borne in mind that, in order to confirm a harmful risk of F treatment, more direct markers should be analyzed, such as endothelial function. Furthermore, other markers associated with an increase in cardiovascular risk, such as apolipoprotein B-100 serum levels [30], LDLox and TBARS, were not altered by F treatment (Table 3).

Regarding glucose metabolism, although no effects were observed on glucose and insulin levels, HOMA-IR and R-QUICKI indexes (Table 3) or OGTT (*data not shown*) by F treatment, a significant reduction of HbA1c, which is considered a plasmatic indicator of long-term glucose levels, was found in both STD+F and CAF+F groups (Table 3). These results are consistent with some studies in which soluble fiber reduced HbA1c levels in rat models of obesity and diabetes [31], indicating a beneficial effect of fiber on glucose control.

3.5. F supplementation improves intestinal health and reduces endotoxaemia

CAF+F animals presented higher stool fresh weight (Student's t-test, $P<.05$), which indicates beneficial effects of F on constipation (*data not shown*). These results concur with the reported beneficial effect of dietary fiber and dried plums on intestinal function mediated by the increase of bowel movement frequency and stool weight [32,33]. Furthermore, F treatment produced an increase in cecal weight (%) in both dietary groups (Table 4). This increase has been previously allocated to a rise in epithelial cell proliferation as a result of the trophic effect of SCFAs produced during fiber fermentation in the cecum by microbiota [34]. The length of the intestines is considered a good indicator of intestinal inflammation, as the shorter their length, the greater the intestinal inflammation [35–37]. In this respect, F treatment also increased the small intestine length:weight ratio in both dietary groups (Table 4), suggesting a decrease in the inflammatory state of this tissue

Table 3
Serum, liver, and fecal parameters related to lipid and glucose metabolism, systemic inflammation and oxidative system in rats fed standard diet (STD) or cafeteria diet (CAF) and treated with vehicle (VH) or fiber (F)

	STD+VH	STD+F	CAF+VH	CAF+F	ANOVA
Plasmatic parameters					
Total Chol (mg/dL)	108 ± 4.72	100 ± 3.53	107 ± 3.97	111 ± 5.13	-
LDL-c (mg/dL)	45.5 ± 2.05	40.7 ± 1.55	51.3 ± 1.86	58.8 ± 4.14	D, DxT
HDL-c (mg/dL)	97.1 ± 3.23	86.7 ± 3.37	81.1 ± 4.03	66.5 ± 2.58	D, T
Triglycerides (mg/dL)	83.7 ± 9.11	72.8 ± 10.91	232 ± 17.62	268 ± 30.98	D
NEFAs (mmol/L)	0.45 ± 0.06	0.38 ± 0.03	0.49 ± 0.04	0.45 ± 0.03	-
ApoB-100 (mg/mL)	2.57 ± 0.24	2.13 ± 0.24	2.66 ± 0.22	2.52 ± 0.27	-
LDLox (ng/mL)	20.1 ± 2.04	19.8 ± 1.80	26.8 ± 3.78	20.2 ± 0.78	-
TBARS (μmol/L)	13.0 ± 2.16	19.6 ± 3.24	24.3 ± 3.78	28.4 ± 2.22	D
Glucose (mg/dL)	111 ± 2.46	105 ± 3.07	123 ± 3.13	122 ± 3.50	-
Insulin (ng/mL)	1.02 ± 0.11	0.93 ± 0.07	1.81 ± 0.14	1.55 ± 0.17	D
HOMA-IR	6.35 ± 0.95	6.05 ± 0.53	13.8 ± 1.18	12.4 ± 1.66	D
R-QUIKI	0.34 ± 0.01	0.34 ± 0.01	0.29 ± 0.005	0.30 ± 0.01	D
HbA1c (μg/mL)	27.6 ± 1.30	21.6 ± 1.30	28.0 ± 1.10	24.6 ± 1.60	T
MCP-1 (ng/mL)	6.30 ± 0.35	6.92 ± 0.40	8.25 ± 0.36	8.18 ± 0.15	D
Hepatic parameters					
Liver (g)	11.4 ± 0.50	10.2 ± 0.30	14.6 ± 0.60	14.9 ± 0.40	D
Liver (%)	4.13 ± 0.08	4.02 ± 0.09	4.05 ± 0.06	4.39 ± 0.07	DxT
Total Lipid (mg)	382 ± 20	343 ± 18	673 ± 49	852 ± 92	D, DxT
Lipid (mg/g)	33.7 ± 1.7	33.6 ± 1.4	49.9 ± 4.6	58.5 ± 5.3	D
Total Chol (mg)	25.0 ± 1.5	20.8 ± 1.2	53.0 ± 7.5	70.6 ± 6.8	D, DxT
Chol (mg/g)	2.21 ± 0.14	2.06 ± 0.12	4.19 ± 0.41	4.83 ± 0.36	D
Total Triglycerides (mg)	62.8 ± 4.5	53.5 ± 4.2	163 ± 27	187 ± 19	D
Triglycerides (mg/g)	5.53 ± 0.41	5.29 ± 0.40	12.5 ± 1.4	12.8 ± 1.0	D
Fecal parameters					
Lipid content (mg/g feces)	18 ± 1.48	16.7 ± 0.46	26.8 ± 1.80	24.3 ± 2.87	D
Lipid content (mg)	70.8 ± 7.21	58.1 ± 3.65	35.7 ± 4.70	35.5 ± 4.62	D
Lipid absorption (g)	1.22 ± 0.05	1.13 ± 0.04	3.90 ± 0.18	4.33 ± 0.26	D

Wistar rats supplemented with fiber (F) or vehicle (VH) and fed a cafeteria (CAF) diet or standard (STD) diet for eight weeks. Chol; cholesterol, NEFAS; non-esterified fatty acid, ox-LDL; oxidized low-density lipoprotein, HbA1c; glycated hemoglobin, MCP-1; monocyte chemoattractant protein-1. Data are given as means ± SEM. D, diet effect; T, fiber treatment effect; DxT, diet-treatment interaction (Two-way ANOVA test, $P<.05$).
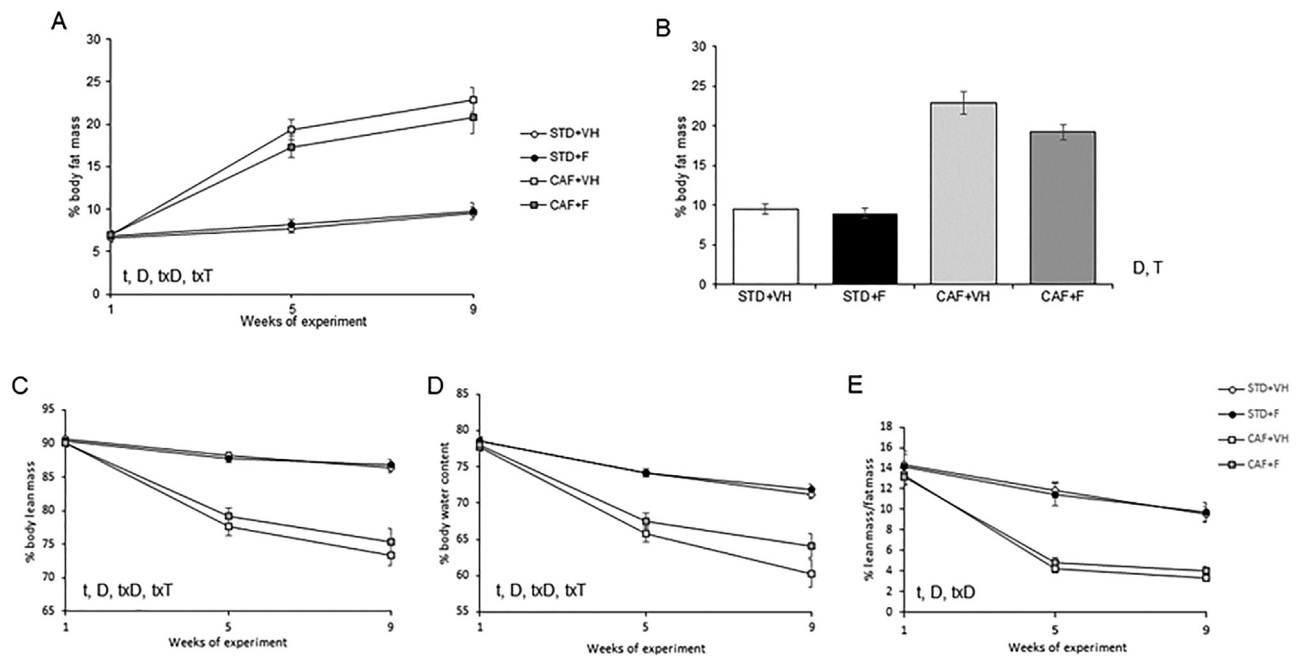

Fig. 4. Body composition parameters of rats fed a standard (STD) or cafeteria (CAF) diet and treated with vehicle (VH) or fiber (F). (A) Body fat mass evolution, (B) Body fat mass at the end of the study, (C) Body lean mass evolution, (D) Body water content evolution, (E) Ratio between body lean and fat mass evolution. The data are presented as the mean ± SEM. D, the effect of the diet; T, the effect of the fiber treatment; t, the effect of the time; txD, the interaction between time and diet; txT, the interaction between time and treatment (Two-way ANOVA and RMs ANOVA, $P<.05$).
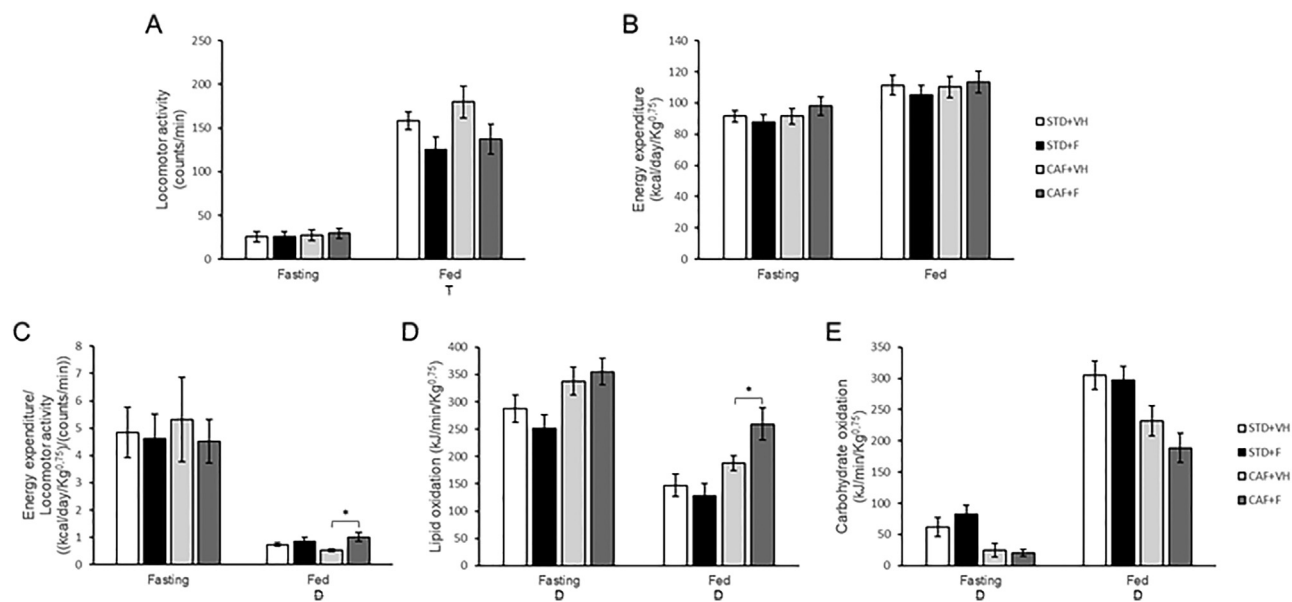
Fig. 5. Indirect calorimetry and locomotor activity of rats fed standard (STD) or cafeteria (CAF) diet and treated with vehicle (VH) or fiber (F). (A) Locomotor activity. (B) Energy expenditure. (C) Ratio between energy expenditure and locomotor activity. (D) Lipid oxidation. (E) Carbohydrate oxidation. The data are presented as the mean ± SEM. D, the effect of the diet; T, the effect of the fiber treatment; DxT, the interaction between diet and treatment (Two-way ANOVA, *P*<.05). *Significantly different between groups (Student's t-test, *P*<.05).

Table 4
Intestinal parameters and endotoxemia in rats fed standard diet (STD) or cafeteria diet (CAF) and treated with vehicle (VH) or fiber (F)

	STD+VH	STD+F	CAF+VH	CAF+F	ANOVA
Cecum (g)	5.31 ± 0.38	5.78 ± 0.28	3.44 ± 0.23	3.91 ± 0.17	D
Cecum (%)	1.82 ± 0.07	2.29 ± 0.11	0.99 ± 0.07	1.20 ± 0.06	D, T
Intestine (cm)	120 ± 1	118 ± 1	122 ± 2	121 ± 2	-
Intestine (cm/g)	15.5 ± 0.5	16.6 ± 0.6	13.3 ± 0.6	14.4 ± 0.4	D, T
Colon (cm)	21.4 ± 0.4	19.4 ± 0.6	18.3 ± 0.6	18.7 ± 0.4	D, DxT
Colon (cm/g)	14.6 ± 0.4	15.3 ± 0.6	16.0 ± 0.9	16.5 ± 0.6	-
MPO (U/mg tissue)	0.07 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	T
LPS (nM)	41.9 ± 5.61	53.2 ± 8.8	153.50 ± 27.1	62 ± 10.8	D, DxT

Wistar rats supplemented with fiber (F) or vehicle (VH) and fed a cafeteria (CAF) diet or standard (STD) diet for 8 weeks. MPO; myeloperoxidase, LPS; lipopolysaccharides. Cecum weight is expressed as absolute weight (g) and relative weight (%), calculated according to the formula (100*tissue weight/body weight) and expressed as percentage of body weight. Data are given as mean ± SEM. D, diet effect; T, fiber treatment effect; DxT, diet-treatment interaction (Two-way ANOVA test, *P*<.05).

[35–37]. In contrast, F treatment decreased colon length in the STD-fed group (Student's t-test, *P*<.05). Although this result could suggest increased colon inflammation as a result of F treatment, it should be borne in mind that this effect was only observed in animals fed the STD diet, where no external inducers of intestinal inflammation were observed, as indicated by MCP1 circulant levels (Table 3). In addition, F treatment produced a significant reduction of MPO activity in both STD and CAF groups (Table 4), suggesting that F treatment induces a decrease in inflammation at the colon level in both groups.

Furthermore, F treatment showed a clear reduction in LPS serum levels in CAF-fed animals (Student's t-test, *P* *P*<.05) (Table 4). These results are consistent with several *in vivo* [38] and human intervention studies [25], and reinforce that fermentable fiber might alter the composition and/or activity of intestinal microbiota, leading to a decrease in intestinal permeability and LPS plasma levels [38]. Interestingly, the body fat of animals was positively correlated with LPS levels and negatively correlated with cecum weight and intestine length (Fig. 2). The main consequence of intestinal barrier dysfunction is the entry of toxins, including LPS, causing metabolic endotoxemia [39,40]. Moreover, the results

of numerous studies suggest that changes in the function of the intestinal barrier and intestinal inflammation are associated with, and could induce, metabolic alterations, including systemic inflammation, dysregulation of body weight or changes in glucose homeostasis [41]. Overall, our results suggest that F has beneficial effects on intestinal inflammation, permeability and health, and that the clear effect of F treatment on endotoxemia in CAF-fed animals could be associated with the beneficial effects observed on body weight, fat mass and glucose metabolism.

3.6. Involvement of metabolites derived from F supplementation in adiposity, lipid metabolism, and intestinal health: SCFAs, phenolic acids, and vitamins

Metabolomic profiles of urine and, to a lesser extent, serum samples showed alterations in metabolites that could be regarded as direct or indirect biomarkers of the F intervention (Table 2). Urinary levels of 5-(hydroxymethyl-2-furoyl)glycine were increased as a consequence of the F treatment in both study groups (*P*<.001), in line with previous works reporting that furfural-related metabolites are normally excreted after the consumption of fiber-rich

Table 5
Significantly changed microbiota between rats fed standard diet (STD) or cafeteria diet (CAF) and treated with vehicle (VH) or fibre (F)

Taxonomic level	STD+VH	STD+F	CAF+VH	CAF+F	ANOVA
Firmicutes	79.4 ± 2.62	78.3 ± 4.08	55.3 ± 4.57	51.5 ± 6.99	D
Lactobacillaceae	1.61 ± 0.26	4.45 ± 0.67	10.1 ± 1.19	15.6 ± 5.39	D, T
Lactobacillus	1.61 ± 0.26	4.45 ± 0.66	10.1 ± 1.91	15.6 ± 5.38	D, T
Christensenellaceae	0.010 ± 0.002	0.018 ± 0.004	0.045 ± 0.012	0.015 ± 0.005	D, DxT
Ruminococcaceae					
Ruminococcus	1.16 ± 0.10	1.38 ± 0.19	0.58 ± 0.21	1.48 ± 0.30	T
Bacteroidetes	18.9 ± 2.62	19.1 ± 3.71	35 ± 4.18	38.8 ± 5.23	D
Porphyromonadaceae	0.176 ± 0.032	0.347 ± 0.101	1.66 ± 0.38	4.00 ± 1.04	D, T
Parabacteroides	0.1175 ± 0.032	0.346 ± 0.101	1.66 ± 0.38	4.00 ± 1.04	D, T
Paraprevotellaceae	$2.06 \cdot 10^{-3} \pm 8.23 \cdot 10^{-4}$	0.028 ± 0.016	0.059 ± 0.030	0.007 ± 0.003	DxT
Proteobacteria	0.20 ± 0.05	0.39 ± 0.14	2.56 ± 0.88	2.42 ± 0.46	D
Desulfovibrionaceae					
Desulfovibrio	$3.30 \cdot 10^{-4} \pm 1.78 \cdot 10^{-4}$	$0.002 \pm 9.63 \cdot 10^{-4}$	0.004 ± 0.001	$0.001 \pm 3.58 \cdot 10^{-4}$	DxT

Wistar rats supplemented with fibre (F) or vehicle (VH) and fed a cafeteria (CAF) diet or standard (STD) diet for 8 weeks. **Phylum;** Family; Genus.

foods [42]. We also observed an increase of SCFAs (butyrate, P=.049; gamma-aminobutyrate, P=.015), which are produced by bacterial fermentation of non-digestible fibers [43]. Furthermore, urinary levels of multiple phenolic acids and flavonoids were significantly increased in the CAF group as a direct effect of the F treatment (P<.05), but the opposite trend (not reaching statistical significance, P<.1) was observed in STD-fed animals. Similarly, F intake also provoked the reduction of various serum vitamins in the STD group (*e.g.*, thiamine, P=.016; α-tocopherol, P=.009), whereas their concentrations were slightly increased in the CAF-fed group. These apparently contradictory results could be attributed to the fact that the plum employed in this study is expected to increase the supply of these plant-related bioactives in animals fed with the CAF diet, which is enriched in unhealthy foods. On the other hand, F supplementation could reduce the absorption of polyphenols and vitamins that are naturally present in the cereal-containing chow that was employed in the STD group, thus decreasing their circulating levels, in line with previous studies [44].

As a result of this differential metabolomics effect of F between STD-fed and CAF-fed animals, correlation analyses showed opposite directions of association according to the diet group (Figure 3). SCFAs and phenolic acids were positively correlated with intestinal health-related parameters in CAF-fed animals (*e.g.*, cecum relative weight with butyric acid, DHCA, DHCA-3S and 3-HPHPA, and 24-h feces weight with CA derivatives and other phenolics), whereas the opposite trend was observed in the STD group (*e.g.*, 24-h feces weight with aminobutyric acid and VA-S). Similarly, these metabolite classes were significantly correlated with several adiposity variables (*e.g.*, positive correlation with fat mass, weight gain and EWAT in STD animals, negative correlation with total liver cholesterol in CAF animals). Therefore, these results could suggest that the beneficial effects of F treatment on obesity, liver lipid metabolism and intestinal health are mediated, at least in part, by SCFAs and phenolic acids.

In turn, we observed an increased abundance of the *Ruminococcus* genus in response to the dietary intervention with F (Table 5), which was positively correlated with urinary levels of phenolic acids and with fresh stool weight in CAF-fed animals (Fig. 3). In this respect, various authors have previously described an increase of these saccharolytic species when adding fermentable fiber or carbohydrates to the diet [45], bacteria that are responsible for the first steps of the microbiota-derived degradation and fermentation of carbohydrates. Notably, *Ruminococcus* species belong to the *Clostridium* cluster IV, which are also essential for the bioavailabil-

ity of most polyphenols by driving C-ring cleavage reactions that lead to the production of simpler phenolic acids [46].

Within this tangled interplay between phenolic compounds and the gut microbiota, F treatment also altered the expression of genes involved in tight junction functioning, downregulating the *protein kinase cAMP-activated catalytic subunit beta, radixin* and *protein kinase C, iota* (Table S1). This is in line with previous studies reporting that dietary phenolics can regulate the synthesis, expression, and redistribution of tight junction proteins [47]. Tight junctions are the principal determinants of epithelial paracellular permeability and help to regulate epithelial barrier properties [46]. They are organized by specific interactions between a wide spectrum of proteins, including the integral role of the claudin family of transmembrane proteins [47]. The results obtained in our study concur with previous studies reporting the expression of genes related to tight junction processes in PBMCs under several conditions [48,49]. In particular, it should be noted that the interaction of LPS with human small intestinal lamina propria fibroblasts has been shown to favor PBMC adhesion through the production of adhesion molecules [50]. Furthermore, obesity induced by the intake of a high-fat diet triggers gut hyperpermeability through tissue-specific claudin switching in the gut epithelium of obese rather than lean subjects [49], in line with the upregulated expression levels of claudin observed in our study.

3.7. F modulates proteolysis and circulating amino acid levels

The intervention with F reduced serum and urinary amino acids towards similar levels to those detected in STD-fed rats (Table 2, P<.05). These amino acids were positively correlated with various adiposity-related parameters (*e.g.*, MWAT, EWAT and body fat mass with proline) in CAF animals (Fig. 3), thus evidencing the great importance of their proper homeostasis in regulating the metabolic complications promoted by this obesogenic diet. These findings concur with previous studies reporting that F treatment may significantly influence the metabolism of amino acids through different mechanisms, including enhanced hepatic gluconeogenesis [48], decreased proteolysis [49] and increased efficiency of amino acid utilization to maintain protein synthesis [45].

Moreover, serum amino acids (*i.e.*, threonine) were negatively correlated with the cecal abundance of the *Lactobacillus* genus (Fig. 3). F feeding induced an increase in *Lactobacillus* counts, in line with previous studies indicating that dietary fibers provoke important alterations in the gut microbiota composition to-

wards increased saccharolytic species and decreased detrimental proteolytic bacteria [49]. Similarly, Kieffer et al. reported that the abundance of various beneficial bacterial families (including *Lactobacillaceae*) showed negative correlations with liver nitrogenous metabolites (*e.g.*, amino acids, urea) in mice fed a high-fat diet and supplemented with resistant starch [45]. Altogether, these results seem to indicate that F treatment in CAF-fed rats could impact amino acid metabolism by modulating the microbiota composition and by decreasing the proteolysis rate. Supporting this hypothesis, transcriptomic data in PBMCs also revealed the downregulation of several genes involved in the proteasome in F-treated animals (Table S1). These results concur with previous studies reporting that obesity is associated with an increase in skeletal muscle atrophy and a decrease in myofibrillar proteins as a result of ubiquitin-mediated overactivation of the proteasome pathway [50], whereas F treatment could decrease the muscle atrophy caused by the CAF diet. Furthermore, this altered protein turnover was also reflected in a higher serum creatine:creatinine ratio in fiber-treated CAF-fed animals (Table 2), in line with previous works [51].

As expected, serum levels of 1-methylhistidine and 3-methylhistidine increased in CAF-fed animals compared with the STD group, as methylhistidine metabolites are well-known biomarkers of meat intake (*e.g.*, pâté and bacon supplied in the CAF diet) [28]. However, this increase was surprisingly sharper in response to the F treatment in the CAF group. Similarly, Korsholm et al. found that resveratrol treatment in men with MetS induced the accumulation of 3-methylhistidine and N-acetyl-3-methylhistidine in blood samples [52]. In particular, we observed that 1-methylhistidine concentrations correlated negatively with adiponectin levels and positively with liver weight in CAF-fed animals, whereas 3-methylhistidine negatively correlated with EWAT in STD animals (Fig. 3), which evidences the great impact of these amino acid derivatives on adiposity and liver health. Accordingly, deeper studies are needed to determine the exact effect of fibre on skeletal muscle function.

3.8. Impact of F on the crosstalk between circulating amino acids and intestinal health

The intake of fibres induced a significant decrease of serum and urinary levels of phenylalanine (*i.e.*, phenyllactic acid, phenylacetylglycine, phenylacetylglutamine) and tryptophan (*i.e.*, indolepropionic acid) derivatives in CAF-fed animals (Table 2, *P*<.05), in line with previously published studies [45,49,53,54]. These are common host-microbial co-metabolites produced by specific bacterial strains most of which are well-known uremic toxins. Our metagenomic results did not show any significant change in bacterial species that are recognized to be involved in aromatic amino acid metabolism, nor significant correlations between metabolomic and metagenomic data. However, serum amino acids and derivatives (*e.g.*, proline, phenyllactic acid) were positively associated with plasma LPS and negatively correlated with cecum weight in the CAF group (Fig. 3), thus corroborating the pivotal role of the interplay between the gut microbiota and amino acids in modulating endotoxemia and intestinal health.

Ergothioneine is a histidine metabolite that is usually acquired from the diet and shows antioxidant properties. Interestingly, we observed that the CAF diet lowered ergothioneine concentrations both in urine and serum samples, whereas F treatment tended to restore the original content of this compound in urine (*P*=.037, Table 2). Nonetheless, the mechanisms behind these metabolomic changes and consequences for health outcomes are uncertain, since no significant correlations were noticed with other biometrical, biochemical or metagenomic variables.

4. Conclusions

In conclusion, the results of our study demonstrate that supplementation over 8 weeks with a mixture of highly fermentable fibers at a dose extrapolable to humans reduced the adiposity of animals, improved intestinal health and had a clear effect on endotoxemia mainly in CAF-fed animals. The treatment also decreased liver cholesterol levels in STD-fed animals and reduced HbA1c blood levels in both STD- and CAF-fed animals. The integrative approach applied in this study evidenced that the major drivers in defining phenotypic status were biometrical and biochemical variables together with metabolomic data. Furthermore, correlation results suggest that the effect of F on obesity could be mediated by an increase of energy expenditure and lipid oxidation. Gene expression analysis in PBMCs is in accordance with some of the beneficial effects observed by the F treatment, showing significant changes in thermogenic, tight junction and proteolysis pathways. Metabolomics results evidenced an accumulation of urinary phenolic compounds and short-chain fatty acids as a consequence of F supplementation, as well as decreased levels of amino acids in serum and urine. This reinforces the central involvement of the gut microbiota and protein metabolism in the F-mediated ameliorating effects on the CAF-fed animals. Altogether, the present study highlights the added value of using integrative multi-omics approaches with the aim of obtaining a deeper understanding of the phenotypic effects and the underlying molecular mechanisms behind the beneficial effects of dietary interventions against obesity and related disorders. Further randomized controlled clinical trials focused on the effectiveness of F against obesity and its metabolic-related disorders would strongly contribute to shedding more light on this issue.

Author contributions

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2022.109184.

References

[1] McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. Clin Dermatol 2018;36:14–20. doi:10.1016/j.clindermatol.2017.09.004.
[2] Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep 2018;20. doi:10.1007/s11906-018-0812-z.
[3] Dabke K, Hendrick G, Devkota S. The gut microbiome and metabolic syndrome. J Clin Invest 2019;129:4050–7. doi:10.1172/JCI129194.
[4] Chen JP, Chen GC, Wang XP, Qin L, Bai Y. Dietary fiber and metabolic syndrome: A meta-analysis and review of related mechanisms. Nutrients 2018;10. doi:10.3390/nu10010024.
[5] de la Iglesia R, Loria-Kohen V, Zulet MA, Martinez JA, Reglero G, de Molina AR. Dietary strategies implicated in the prevention and treatment of metabolic syndrome. Int J Mol Sci 2016;17. doi:10.3390/ijms17111877.
[6] Barber TM, Kabisch S, Pteiffer AFHWMO. The health benefits of dietary fibre. Nutrients 2020;12:3209–26.
[7] Bozzetto L, Costabile G, Della Pepa G, Ciciola P, Vetrani C, Vitale M, et al. Dietary fibre as a unifying remedy for the whole spectrum of obesity-associated cardiovascular risk. Nutrients 2018;10. doi:10.3390/nu10070943.
[8] Caimari A, del Bas JM, Boqué N, Crescenti A, Puiggròs F, Chenoll E, et al. Heat-killed Bifidobacterium animalis subsp. Lactis CECT 8145 increases lean mass and ameliorates metabolic syndrome in cafeteria-fed obese rats. J Funct Foods 2017;38:251–63. doi:10.1016/j.jff.2017.09.029.
[9] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659–61. doi:10.1096/FJ.07-9574LSF.
[10] Stephen AM, Champ MMJ, Cloran SJ, Fleith M, Van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: Current state of knowledge on definitions. sources, recommendations, intakes and relationships to health 2017;30. doi:10.1017/S095442241700004X.
[11] Crescenti A, del Bas JM, Arola-Arnal A, Oms-Oliu G, Arola L, Caimari A. Grape seed procyanidins administered at physiological doses to rats during preg-

[12] Caimari A, Crescenti A, Puiggròs F, Boqué N, Arola L, del Bas J. The intake of a high-fat diet and grape seed procyanidins induces gene expression changes in peripheral blood mononuclear cells of hamsters: capturing alterations in lipid and cholesterol metabolism. Genes Nutr 2015;10:438–48.
[13] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–19. doi:10.1007/BF00280883.
[14] Perseghin G, Caumo A, Caloni M, Testolin G. Incorporation of the Fasting Plasma FFA Concentration into QUICKI Improves Its Association with Insulin Sensitivity in Nonobese Individuals. vol. 86. 2001.
[15] Alexandre V, Even PC, Larue-Achagiotis C, Blouin JM, Blachier F, Benamouzig R, et al. Lactose malabsorption and colonic fermentations alter host metabolism in rats. Br J Nutr 2013;110:625–31. doi:10.1017/S0007114512005557.
[16] Sung J, Morales W, Kim G, Pokkunuri V, Weitsman S, Rooks E, et al. Effect of repeated Campylobacter jejuni infection on gut flora and mucosal defense in a rat model of post infectious functional and microbial bowel changes. Neuro-gastroenterol Motil 2013;25:529–37. doi:10.1111/nmo.12118.
[17] Caimari A, del Bas JM, Crescenti A, Arola L. Low doses of grape seed procyanidins reduce adiposity and improve the plasma lipid profile in hamsters. Int J Obes (Lond) 2013;37:576–83. doi:10.1038/ijo.2012.75.
[18] González-Domínguez R, Urpi-Sarda M, Jáuregui O, Needs PW, Kroon PA. Andrés-Lacueva C. Quantitative Dietary Fingerprinting (QDF)-A Novel Tool for Comprehensive Dietary Assessment Based on Urinary Nutrimetabolomics. J Agric Food Chem 2020;68:1851–61. doi:10.1021/acs.jafc.8b07023.
[19] Palacios-Jordan H, Martín-González MZ, Suárez M, Aragonès G, Mugureza B, Rodríguez MA, et al. The disruption of liver metabolic circadian rhythms by a cafeteria diet is sex-dependent in fischer 344 rats. Nutrients 2020;12. doi:10.3390/nu12041085.
[20] Rohart F, Gautier B, Singh A. Lê Cao KA. mixOmics: an R package for 'omics feature selection and multiple data integration. BioRxiv 2017. doi:10.1101/108597.
[21] Caimari A, Oliver P, Keijer J, Palou A. Peripheral blood mononuclear cells as a model to study the response of energy homeostasis-related genes to acute changes in feeding conditions. Omi A J Integr Biol 2010;14:129–41. doi:10.1089/omi.2009.0092.
[22] Britton KA, Massaro JM, Murabito JM, Kreger BE, Hoffmann U, Fox CS. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. J Am Coll Cardiol 2013;62:921–5. doi:10.1016/j.jacc.2013.06.027.
[23] Artiss JD, Brogan K, Brucal M, Moghaddam M, Jen KLC. The effects of a new soluble dietary fiber on weight gain and selected blood parameters in rats. Metabolism 2006;55:195–202. doi:10.1016/j.metabol.2005.08.012.
[24] Sánchez D, Miguel M, Aleixandre A. Dietary fiber, gut peptides, and adipocytokines. J Med Food 2012;15:223–30. doi:10.1089/jmf.2011.0072.
[25] Parnell JA, Klancic T, Reimer RA. Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. Obesity 2017;25:510–13. doi:10.1002/oby.21763.
[26] Ribot J, Rodríguez AM, Rodríguez E, Palou A. Adiponectin and resistin response in the onset of obesity in male and female rats. Obesity 2008;16:723–30. doi:10.1038/oby.2008.113.
[27] Mika A, Day HEW, Martinez A, Rumian NL, Greenwood BN, Chichlowski M, et al. Early life diets with prebiotics and bioactive milk fractions attenuate the impact of stress on learned helplessness behaviours and alter gene expression within neural circuits important for stress resistance. Eur J Neurosci 2017;45:342–57. doi:10.1111/ejn.13444.
[28] Castellano-Escuder P, González-Domínguez R, Wishart DS, Andrés-Lacueva C, Sánchez-Pla AFOBI. An ontology to represent food intake data and associate it with metabolomic data. Database 2020;2020. doi:10.1093/databa/baaa033.
[29] Surampudi P, Enkhmaa B, Anuurad E, Berglund L. Lipid Lowering with Soluble Dietary Fiber. Curr Atheroscler Rep 2016;18. doi:10.1007/s11883-016-0624-z.
[30] Pires A, Sena C, Seiça R. Dyslipidemia and cardiovascular changes in children. Curr Opin Cardiol 2016;31:95–100. doi:10.1097/HCO.0000000000000249.
[31] Brockman DA, Chen X, Gallaher DD. Hydroxypropyl methylcellulose, a viscous soluble fiber, reduces insulin resistance and decreases fatty liver in Zucker Diabetic Fatty rats. Nutr Metab 2012;9. doi:10.1186/1743-7075-9-100.
[32] Bosaeus I. Fibre effects on intestinal functions (diarrhoea, constipation and irritable bowel syndrome). Clin Nutr Suppl 2004;1:33–8. doi:10.1016/j.clnu.2004.09.006.
[33] Attaluri A, Donahoe R, Valestin J, Brown K, Rao SSC. Randomised clinical trial: dried plums (prunes) vs. psyllium for constipation. Aliment Pharmacol Ther 2011;33:822–8. doi:10.1111/j.1365-2036.2011.04594.x.
[34] Knapp BK, Bauer LL, Swanson KS, Tappenden KA, Fahey GC, de Godoy MRC. Soluble fiber dextrin and soluble corn fiber supplementation modify indices of health in cecum and colon of Sprague-Dawley rats. Nutrients 2013;5:396–410. doi:10.3390/NU5020396.
[35] Li N, Liboni K, Fang MZ, Samuelson D, Lewis P, Patel R, et al. Glutamine decreases lipopolysaccharide-induced intestinal inflammation in infant rats. Am J Physiol - Gastrointest Liver Physiol 2004;286:914–21. doi:10.1152/AJPGI.00493.2003/ASSET/IMAGES/LARGE/ZH30060416920007.JPEG.
[36] Qu B, Xin GR, Zhao LX, Xing H, Lian LY, Jiang HY, et al. Testing stem cell therapy in a rat model of inflammatory bowel disease: role of bone marrow

stem cells and stem cell factor in mucosal regeneration. PLoS One 2014;9. doi:10.1371/JOURNAL.PONE.0107891.

[37] Pereira JNB, Murata GM, Sato FT, Marosti AR, Carvalho CR de O, Curi R. Small intestine remodeling in male Goto-Kakizaki rats. Physiol Rep 2021;9. doi:10.14814/PHY2.14755.

[38] Cani PD, Possemiers S, Van De Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–103. doi:10.1136/gut.2008.165886.

[39] Laugerette F, Vors C, Géloën A, Chauvin MA, Soulage C, Lambert-Porcheron S, et al. Emulsified lipids increase endotoxemia: Possible role in early post-prandial low-grade inflammation. J Nutr Biochem 2011;22:53–9. doi:10.1016/j.jnutbio.2009.11.011.

[40] Neves AL, Coelho J, Couto L, Leite-Moreira A, Roncon-Albuquerque R. Metabolic endotoxemia: A molecular link between obesity and cardiovascular risk. J Mol Endocrinol 2013;51. doi:10.1530/JME-13-0079.

[41] Raybould HE. Gut microbiota, epithelial function and derangements in obesity. J Physiol 2012;590:441–6. doi:10.1113/jphysiol.2011.222133.

[42] Hardt-Stremayr M, Mattioli S, Greilberger J, Stiegler P, Matzi V, Schmid MG, et al. Determination of metabolites of 5-hydroxymethylfurfural in human urine after oral application. J Sep Sci 2013;36:670–6. doi:10.1002/jssc.201200768.

[43] Verbeke KA, Boobis AR, Chiodini A, Edwards CA, Franck A, Kleerebezem M, et al. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. Nutr Res Rev 2015;28:42–66. doi:10.1017/S0954422415000037.

[44] Palafox-Carlos H, Ayala-Zavala JF, González-Aguilar GA. The Role of Dietary Fiber in the Bioaccessibility and Bioavailability of Fruit and Vegetable Antioxidants. J Food Sci 2011;76. doi:10.1111/j.1750-3841.2010.01957.x.

[45] Kieffer DA, Piccolo BD, Marco ML, Kim EB, Goodson ML, Keenan MJ, et al. Mice fed a high-fat diet supplemented with resistant starch display marked shifts in the liver metabolome concurrent with altered gut bacteria. J Nutr 2016;146:2476–90. doi:10.3945/jn.116.238931.

[46] Cortés-Martín A, Selma MV, Tomás-Barberán FA, González-Sarrías A, Espín JC. Where to look into the puzzle of polyphenols and health? The postbiotics and gut microbiota associated with human metabotypes. Mol Nutr Food Res 2020;64. doi:10.1002/mnfr.201900952.

[47] Peron G, Hidalgo-Liberona N, González-Domínguez R, Garcia-Aloy M, Guglielmetti S, Bernardi S, et al. Exploring the molecular pathways behind the effects of nutrients and dietary polyphenols on gut microbiota and intestinal permeability: a perspective on the potential of metabolomics and future clinical applications. J Agric Food Chem 2020;68:1780–9. doi:10.1021/acs.jafc.9b01687.

[48] Kawaguchi M, Nishikoba N, Shimamoto S, Tomonaga S, Kohrogi R, Yamauchi Y, et al. Feeding the outer bran fraction of rice alters hepatic carbohydrate metabolism in rats. Nutrients 2020;12. doi:10.3390/nu12020430.

[49] Jackson MI, Jewell DE. Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. Gut Microbes 2019;10:298–320. doi:10.1080/19490976.2018.1526580.

[50] Rasool S, Geetha T, Broderick TL, Babu JR. High fat with high sucrose diet leads to obesity and induces myodegeneration. Front Physiol 2018;9. doi:10.3389/fphys.2018.01054.

[51] Ross AB, Pere-Trépat E, Montoliu I, Martin FPJ, Collino S, Moco S, et al. A whole-grain-rich diet reduces urinary excretion of markers of protein catabolism and gut microbiota metabolism in healthy men after one week. J Nutr 2013;143:766–73. doi:10.3945/jn.112.172197.

[52] Korsholm AS, Kjær TN, Ornstrup MJ, Pedersen SB. Comprehensive metabolomic analysis in blood, urine, fat, and muscle in men with metabolic syndrome: A randomized, placebo-controlled clinical trial on the effects of resveratrol after four months' treatment. Int J Mol Sci 2017;18. doi:10.3390/ijms18030554.

[53] Kieffer DA, Piccolo BD, Vaziri ND, Liu S, Lau WL, Khazaeli M, et al. Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. Am J Physiol - Ren Physiol 2016;310:F857–71. doi:10.1152/ajprenal.00513.2015.

[54] Sattari Najafabadi Z, Skau Nielsen T, Skou Hedemann M. Dietary protein source and butyrylated high-amylose maize starch included in a high-protein diet determines the urinary metabolome of rats. Int J Food Sci Nutr 2019;70:255–66. doi:10.1080/09637486.2018.1499711.