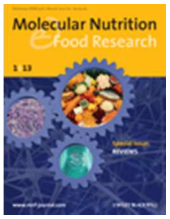

Gut microbiota interacts with markers of adipose tissue browning, insulin action and plasma acetate in morbid obesity

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Complete List of Authors:	Moreno-Navarrete, José María Serino, Matteo Blasco-Baque, Vincent Azalbert, Vincent Barton, Richard Cardellini, Marina Latorre, Jessica Ortega, Francisco; Institut d'Investigació Biomèdica de Girona (IdIBGi), Service of Diabetes, Endocrinology and Nutrition (UDEN); José Ortega, Sabater-Masdeu, Mònica Burcelin, R Dumas, ME Ricart, Wifredo Federici, Massimo Fernández-Real, José Manuel
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Gut microbiota interacts with markers of adipose tissue browning, insulin action and plasma acetate in morbid obesity

José María Moreno-Navarrete[1*], Matteo Serino[2], Vincent Blasco-Baque[2], Vincent Azalbert[2], Richard Barton[3], Marina Cardellini[4], Jèssica Latorre[1], Francisco Ortega[1], Mònica Sabater-Masdeu[1], Rémy Burcelin[2], Marc Dumas[3], Wifredo Ricart[1], Massimo Federici[4], José Manuel Fernández-Real[1*]

[1]Department of Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomèdica de Girona (IdIBGi), CIBEROBN (CB06/03/010) and Instituto de Salud Carlos III (ISCIII), Girona, Spain.

[2]Institut National de la Santé et de la Recherche Médicale (Inserm), U1048, I2MC, Toulouse, France

[3]Faculty of Medicine, Department of Surgery and Cancer, Division of Computational and Systems Medicine, Imperial College London, Sir Alexander Fleming Building, Exhibition Road, South Kensington, London SW7 2AZ, UK.

[4]Department of Systems Medicine and Center for Atherosclerosis, University of Rome "Tor Vergata", Rome, Italy.

Short title:: Ruminococcaceae and adipose tissue browning

Corresponding author and person to whom reprint requests should be addressed:
J.M. Moreno-Navarrete, PhD e-mail: jmoreno@idibgi.org
J.M. Fernández-Real, M.D. Ph.D e-mail: jmfreal@idibgi.org
Section of Diabetes, Endocrinology and Nutrition
Hospital of Girona "Dr Josep Trueta"
Carretera de França s/n, 17007, Girona, SPAIN.
Phone: 34-972-94 02 00
Fax: 34-972-94 02 70

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; M, insulin action value measured by the euglycemic hyperinsulinemic clamp; RA, relative abundance; RU, relative units of gene expression; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Keywords: adipose tissue, insulin sensitivity, metabolome, microbiota, obesity.

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Abstract

Scope: To examine the potential relationship among gene expression markers of adipose tissue browning, gut microbiota and insulin sensitivity in humans.

Methods and results: Gut microbiota composition and gene markers of browning were analysed in subcutaneous (SAT) and visceral (VAT) adipose tissue from morbidly obese subjects (n=34). Plasma acetate was measured through ^{1}H NMR and insulin sensitivity using euglycemic hyperinsulinemic clamp. Subjects with insulin resistance showed an increase in the relative abundance (RA) of the phyla Bacteroidetes and Proteobacteria while RA of Firmicutes was decreased. In all subjects, Firmicutes RA was negatively correlated with HbA_{1c} and fasting triglycerides, whereas Proteobacteria RA was negatively correlated with insulin sensitivity. Firmicutes RA was positively associated with markers of brown adipocytes (*PRDM16*, *UCP1* and *DIO2*) in SAT, but not in VAT. Multivariate regression analysis indicated that Firmicutes RA contributed significantly to SAT *PRDM16*, *UCP1* and *DIO2* mRNA variance after controlling for age, BMI, HbA_{1c} or insulin sensitivity. Interestingly, Firmicutes RA, specifically those bacteria belonging to Ruminococcaceae family, was positively associated with plasma acetate levels, which were also linked to SAT *PRDM16* mRNA and insulin sensitivity.

Conclusion: Gut microbiota composition is linked to adipose tissue browning and insulin action in humans, possibly through circulating acetate.

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Introduction

The human gut microbiota is composed of 100 trillion microbes that exist in a close symbiotic relationship with human cells, and contain at least 150 times more genes than the whole human genome [1,2]. The metabolic state of the host, diet and medication exert significant effects on the abundance and the composition of the gut microbiota. Experiments in rodents demonstrated that the microbiota can modulate both energy balance (weight gain and loss) and energy stores (fat mass) through the production or the secretion of specific molecules (such as short-chain fatty acids) [3,4].

The dominant phyla in the gut are Firmicutes (~60–65%), Bacteroidetes (~20–25%), Proteobacteria (~5–10%), and Actinobacteria (~3%), which together constitute over 97% of the gut microbe population [4]. In rodents, obesity is associated with an increase in the relative size of the Firmicutes versus Bacteroidetes populations in the gut [5], and a decrease in the diversity of the microbiota that is due to both weight and diet composition. There is much greater variability in human studies. Some studies report a similar increase in the ratio of Firmicutes / Bacteroides, as well as a decrease in the gut biodiversity in obese humans, but there are also numerous studies reporting contradictory findings in obese *vs.* lean humans [6,7]. A recent study demonstrated that cold exposure led to dramatic changes of the microbiota composition, increasing Firmicutes *vs.* Bacteroidetes relative abundance [8]. Interestingly, this shift in microbiota composition is associated to a phenotype of increased capacity of energy extraction, characterized by increased intestinal absorptive surface area and browning of the white adipose tissue [8]. In mice, browning of white adipose tissue prevents obesity-associated metabolic disturbances and insulin resistance [9,10]. In humans, expression

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of markers of browning in subcutaneous adipose tissue has been associated to insulin sensitivity, a relatively healthy obese phenotype and adipose tissue function [11-13].

To the best of our knowledge, the interaction between microbiota and adipose tissue physiology has not been previously investigated in humans. Given the potential importance of adipose tissue inter-relationships with microbiota and insulin action in human obesity, here we aimed to examine the associations among adipose tissue markers of browning (PRDM16, UCP1 and DIO2), insulin sensitivity (SLC2A4 and IRS1) and gut metagenomic microbiota composition in morbidly obese participants. We also studied adipose tissue markers of adipogenesis (ADIPOQ, PPARG) and chronic proinflammatory activity inflammation (TNF and IL6).

Materials and Methods

Recruitment of patients and processing of samples.

The human subject cohort comprised 34 morbidly obese women (n=28) and men (n=6) at the Endocrinology Service of the Hospital Universitari de Girona Dr Josep Trueta (Girona, Spain) included in the FLORINASH project (www.florinash.org). 11 subjects received diabetes, 18 and 9 morbidly obese participants receives hypertension and dyslipemia therapy, respectively. 13 morbidly obese patients have type 2 diabetes, of who 11 received therapy. Pre-established inclusion criteria: all subjects were of Caucasian origin and reported a stable body weight at least three months preceding the study, were free of any infections and antibiotic treatment for one month before inclusion and had no systemic disease. Pre-established exclusion criteria: subjects with

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liver disease, specifically HCV infection and tumor disease, and subjects with thyroid dysfunction were excluded by biochemical workup.

Subcutaneous (SAT) and visceral adipose tissue (VAT) samples were obtained from all subjects during elective gastric bypass surgery and were immediately transported to the laboratory (5-10 min). The handling of tissue was carried out under strictly aseptic conditions. Adipose tissue samples were washed in PBS, cut off with forceps and scalpel into small pieces (100 mg), and immediately flash-frozen in liquid nitrogen before being stored at -80 ºC.

Euglycemic hyperinsulinemic clamp

Insulin action was determined by the euglycemic hyperinsulinemic clamp one week before surgery [14]. After an overnight fast, two catheters were inserted into an antecubital vein, one for each arm, used to administer constant infusions of glucose and insulin, and to obtain arterialized venous blood samples. A 2-h euglycemic hyperinsulinemic clamp was initiated by a two step primed infusion of insulin (80 mU/m2/min for 5 min, 60 mU/m2/min for 5 min) immediately followed by a continuous infusion of insulin at a rate of 40 mU/m2/min (regular insulin; Actrapid, Novo Nordisk, NJ). Glucose infusion began at minute 4 at an initial perfusion rate of 2 mg/kg/min, then was adjusted to maintain plasma glucose concentration at 4.9–5.5 mmol/L. Blood samples were collected every 5 min for determination of plasma glucose and insulin. Insulin sensitivity was assessed as the mean glucose infusion rate during the last 40 min. In the stationary equilibrium, the amount of glucose administered (M) equals the glucose taken by the body tissues and is a measure of overall insulin sensitivity.

Subjects in the upper tertile of glucose infusion rate during the clamp were considered as insulin sensitive.

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All subjects gave written informed consent, validated and approved by the Ethical Committee of the Hospital of Girona "Dr Josep Trueta", after the purpose of the study was explained to them.

Gut microbiota composition

Total DNA was extracted as previously described [15-19]. Briefly, the whole 16S bacterial DNA V2 region was targeted by the 28F-519R primers and analysed by pyrosequencing by the 454 FLX Roche technologies at Research&Testing Laboratory (http://www.researchandtesting.com/, Texas, USA). An average of 5000 sequences was generated per sample. A full description of bioinformatic filters is available at http://www.rtlgenomics.com/docs/Data_Analysis_Methodology.pdf.

Metabolomic analyses

All ^{1}H NMR spectra were acquired using Bruker DRX600 spectrometers (Rheinstetten, Germany) running under TopSpin, with either a 5 mm TXI probe operating at 600.13 MHz or a 5 mm BBI probe operating at 419 600.44 MHz. All runs were carried out using Bruker BACS60 sample handling automation; prior to each run the 90° pulse length was determined and set for the run. The field frequency was locked on D2O as solvent. In all experiments, water suppression was carried out by noise irradiation during the 2 s recycle delay (RD). For all experiments, 128 scans were recorded into 32 K data points with a spectral width of 20 ppm, and an exponential function was applied to the FID prior to the Fourier transformation, which resulted in a line broadening of 0.3 Hz. All plasma NMR spectra were automatically phased, baseline-corrected and referenced to the center of the α-glucose anomeric doublet (δ 5.23) for plasma, using an in-house MatLab (The MathWorks, Natick, Massachusetts) scripts. Baseline and peak alignment quality control was done by individual verification for each spectrum and occasionally a spectrum was manually adjusted.

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RNA expression

RNA purification, gene expression procedures and analyses were carried out, as previously described [13,14]. Briefly, RNA purification was performed using the RNeasy Lipid Tissue Mini Kit (QIAgen, Izasa SA, Barcelona, Spain) and the integrity was checked by the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Gene expression was assessed by real-time PCR using a LightCycler® 480 Real-Time PCR System (Roche Diagnostics SL, Barcelona, Spain), using TaqMan® technology suitable for relative genetic expression quantification. The commercially available and pre-validated TaqMan® primer/probe sets used were detailed elsewhere [13,14].

Analytical methods

Serum glucose, insulin, HOMA-IR, HDL-cholesterol, fasting triglycerides and C-reactive protein concentration measurement were performed as previously described [13,14].

Statistical analyses

Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as mean and SD for Gaussian variables or median and interquartile range. Parameters that did not fulfil normal distribution criteria were Log transformed to improve symmetry for subsequent analyses. The relation between variables was analyzed by simple correlation (Pearson's test and Spearman's test) and multiple regression analyses in a stepwise manner. Levels of statistical significance were set at $p<0.05$.

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Results

Anthropometrical and metabolic parameters are described in Table 1. In obese subjects with insulin resistance, gut Firmicutes relative abundance (RA) was decreased, whereas Bacteroidetes and Proteobacteria RA were increased compared with insulin sensitive subjects (Table 1). These observations were in parallel to reduced SAT *SLC2A4,* (GLUT4), a tendency towards reduced VAT IRS-1 (P=0.06) and reduced SAT *PRDM16* mRNA among insulin resistant subjects (Table 1).

Firmicutes RA was negatively correlated with HbA_{1c} (r= -0.37, p=0.03) and fasting triglycerides (r= -0.35, p=0.04), whereas Proteobacteria RA was positively correlated with HOMA-IR (r= 0.35, p=0.04) and negatively with insulin sensitivity (r=-0.38, p=0.03).

Interestingly, Firmicutes RA was positively correlated with markers of brown adipocytes [*PRDM16* (r=0.42, p=0.01), *UCP1* (r=0.45, p=0.01) and *DIO2* (r=0.38, p=0.03) (Figure 1a-b)] in SAT, but not in VAT [*PRDM16* (r=-0.05, p=0.7), *UCP1* (r=0.02, p=0.9) and *DIO2* (r=-0.04, p=0.8)]. Otherwise, Bacteroidetes RA was negatively correlated with *PRDM16* (r=-0.37, p=0.03), *UCP1* (r=-0.38, p=0.03) and *DIO2* (r=-0.35, p=0.05) mRNA levels (Figure 1c-d).

The associations were specific for browning, because no significant correlations were found between Firmicutes RA and markers of adipogenesis [*ADIPOQ* (r=0.15, p=0.4), *PPARG* (r=-0.07, p=0.6)], insulin sensitivity [*IRS1* (r=0.17, p=0.3) and *SLC2A4* (r=0.13, p=0.4)] or inflammation [TNF (r=-0.16, p=0.4) and IL6 (r=0.21, p=0.3)] in SAT or VAT (*ADIPOQ* (r=-0.16, p=0.4), *PPARG* (r=-0.02, p=0.9), *SLC2A4* (r=0.03, p=0.8), *IRS1* (r=0.24, p=0.1), TNF (r=-0.03, p=0.8) and IL6 (r=-0.18, p=0.4).

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PRDM16 mRNA levels were also negatively correlated with Proteobacteria RA (r=-0.37, p=0.03), but this association did not remain significant after controlling for age, BMI or insulin sensitivity.

Multivariate regression analysis indicated that Firmicutes RA contributed significantly to SAT *PRDM16* and *UCP1* mRNA variance after controlling for age, sex, BMI, insulin sensitivity (*M* value), HbA_{1c}, and diabetes, hypertension or dyslipemia therapy (Table 2). Firmicutes RA also contributed significantly to SAT *DIO2* mRNA variance after controlling for age, sex, insulin sensitivity (*M* value) and HbA_{1c} (Table 2).

To explore the possible mechanism underlying the relationship between microbiota and adipose tissue browning, and considering the effects of circulating acetate on adipocyte browning and fat oxidation [20-24], its major abundance compared to other important gut microbial-derived short-chain fatty acids (propionate and butyrate) [25], and its capacity to transfer from colon into bloodstream [20], circulating acetate levels in plasma were examined. Circulating acetate levels were significantly increased in insulin sensitive obese participants (Figure 2a) in positive correlation with gut Firmicutes RA, SAT *PRDM16* mRNA and insulin sensitivity (Figure 2b-d), and negatively correlated with HOMA-IR and fasting triglycerides (Figure 2e-f).

Finally, within families from firmicutes phylum, only those bacteria from Ruminococcaceae family, which was the most abundant (Figure 2g), were positively correlated with insulin sensitivity (r=0.48, p=0.01), plasma acetate (r=0.52, p=0.005) and PRDM16 mRNA levels (r=0.43, p=0.02), and negatively correlated with fasting triglycerides (r= -0.37, p= 0.04) (Figure 3). Multivariate regression analysis indicated that plasma acetate contributed significantly to SAT *PRDM16* mRNA variance after

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controlling for age, sex, BMI, insulin sensitivity (*M* value) and diabetes, hypertension or dyslipemia therapy, but not after controlling for family Ruminococcaceae RA (Table 3).

Discussion

In the current study, we investigated gut microbial composition and markers of adipose tissue browning in morbidly obese subjects. To the best of our knowledge, no previous studies have explored these associations in humans. The main findings indicate that Firmicutes RA, specifically those bacteria from Ruminococcaceae family, was positively associated with insulin action in parallel to increased markers of browning in SAT. A previous study demonstrated that cold exposure, with its associated increased energy harvest, led to dramatic changes of the microbiota composition in mice, increasing Firmicutes *vs.* Bacteroidetes RA in association with browning of white adipose tissue. These changes resulted in improved insulin sensitivity, increased energy expenditure and fat loss [8]. In humans, this shift in microbial composition has been previously associated with significantly increased energy harvest [26]. A significant reduction in bacterial species from Firmicutes phylum and increased RA of bacterial species from Proteobacteria and Bacteroidetes phyla are known to be associated with insulin resistance and type 2 diabetes [27-33]. Markers of browning (PRDM16 and UCP1) in SAT have been also linked to insulin sensitivity [11-13].

The mechanisms to explain the relationship between Ruminococcaceae RA and markers of browning in SAT remain to be elucidated. Gut microbial-derived SCFAs have been demonstrated to improve insulin sensitivity through the enhancing of fat oxidation and energy expenditure [20,25,34]. Acetate seems to exert a crucial role in brown adipocyte differentiation through the induction of mitochondrial biogenesis [21], leading to

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increased oxygen consumption rate in white and brown adipocytes and skeletal muscle from Otsuka Long-Evans Tokushima Fatty rats [22]; and increased browning of SAT in parallel to increased insulin sensitivity in mice [23]. Acetate administration also resulted in increased expression of browning genes (PRDM16, UCP1 and DIO2) in 3T3-L1 cells and in white adipose tissue from obese diabetic KK-Ay mice in parallel to increased whole-body oxygen consumption [24], leading to enhanced fat oxidation [25,35,36].

In animal models, reduced Firmicutes RA and serum acetate and increased Bacteroidetes RA were concomitant to lower glucose infusion rate during a hyperinsulinemic euglycemic clamp in mice [37]. The recovery of serum acetate levels (using antibiotics or acetate administration) led to improved insulin sensitivity and attenuated adipose tissue macrophage infiltration in adipose tissue [37].

In humans, distal colonic acetate infusion resulted in increased whole-body fat oxidation [28]. Multivariate regression analysis indicated that the association between plasma acetate and SAT *PRDM16* mRNA remained significant after controlling for age, sex, BMI, insulin sensitivity (*M* value) and diabetes, hypertension or dyslipemia therapy, but not after controlling for family Ruminococcaceae RA, suggesting this bacterial family as the most important source of plasma acetate. In agreement with this suggestion, one recent study found acetate biosynthesis to be correlated with Firmicutes RA [38], being those bacteria from Ruminococcaceae family (which was the most abundant) considered as important acetate-producing bacteria in rumen studies [39]. This is the first study, to our knowledge, to describe associations among glucose infusion rate during euglycemic hyperinsulinemic clamp, gut microbiota composition and markers of browning in SAT in humans.

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The absence of association between Firmicutes RA and markers of browning in VAT might be explained by the lower capacity of this fat depot to develop browning [9,40]. The current study extends to humans previous observations in mice regarding gut microbiota composition and adipose tissue browning [8], and suggests a possible mechanism to explain them through increased plasma acetate levels. Further intervention studies exploring the possible role of acetate in adipose tissue browning and insulin sensitivity are required to confirm current findings.

Author contributions

JMM-N researched data, wrote and edited the manuscript; MS, RB, MD, MF researched data, contributed to discussion and reviewed manuscript; VB-B, VA, RBa, MC, JL, FO, MS-M researched data; WR contributed to discussion; JMF-R researched data and wrote and reviewed the manuscript.

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Table 1. Anthropometric, clinical parameters, microbial abundance and SAT gene expressions according to insulin resistance.

	Insulin sensitive	Insulin resistant	*P*
N	14	20	
Age (years)	48.7 ± 11.1	47.4 ± 7.6	0.7
BMI (kg/m^2)	42.8 ± 7.1	45.4 ± 5.6	0.2
Waist circumference (cm)	123.9 ± 10.7	129.1 ± 11.1	0.2
Fasting glucose (mg/dl)	95.5 (87-100.2)	102 (92.5-129.5)	0.02
HbA_{1c} (%)	5.5 ± 0.5	6.4 ± 1.6	0.02
HbA_{1c} (mmol/mol)	37 ± 3.2	46 ± 9.5	0.02
HDL-cholesterol (mg/dl)	48.0 ± 10.1	46.6 ± 14.8	0.7
Fasting triglycerides (mg/dl)	88.5 (66-147)	134 (99.5-165)	0.1
HOMA-IR	1.6 (1.3-2.1)	4.8 (4.1-8.3)	0.03
M (mg/kg/min)	5.8 ± 2.7	2.7 ± 1.3	<0.0001
Firmicutes (%)	64.9 (60.4-78.7)	47.3 (42.1-64.5)	0.001
Bacteroidetes (%)	29.3 (18.8-36.8)	36.7 (27.1-49.7)	0.02
Proteobacteria (%)	1.27 (0.85-2.98)	2.32 (1.99-5.89)	0.03
Actinobacteria (%)	0.45 (0.19-0.76)	0.28 (0.14-0.61)	0.2
Fusobacteria (%)	0.47 (0.11-1.43)	0.67 (0.24-3.21)	0.4
Shannon index	3.34 ± 0.17	3.23 ± 0.33	0.2
SAT *ADIPOQ* (RU)	2.42 ± 0.7	2.37 ± 0.7	0.8
SAT *PPARG* (RU)	0.036 ± 0.01	0.040 ± 0.02	0.6
SAT *SLC2A4* (RU)	0.053 ± 0.02	0.036 ± 0.01	0.04
SAT *IRS1* (RU)	0.0087 ± 0.005	0.0078 ± 0.003	0.4
SAT *TNF* (RU)	0.0039 ± 0.001	0.0046 ± 0.003	0.4
SAT *IL6* (RU) 10^{-4}	5 (3-20)	13 (5-44)	0.2
SAT *PRDM16* (RU)	0.0037 ± 0.001	0.0027 ± 0.001	0.03
SAT *UCP1* (RU) * 10^{-5}	2.5 (2-3)	2 (1-3)	0.4
SAT *DIO2* (RU) * 10^{-4}	6.4 (4-7)	6.9 (3-11.1)	0.4
VAT *ADIPOQ* (RU)	1.53 ± 0.47	1.36 ± 0.5	0.3
VAT *PPARG* (RU)	0.021 ± 0.008	0.020 ± 0.013	0.8
VAT *SLC2A4* (RU)	0.032 ± 0.02	0.023 ± 0.01	0.1
VAT *IRS1* (RU)	0.0087 ± 0.003	0.0069 ± 0.002	0.06
VAT *TNF* (RU)	0.0052 ± 0.002	0.0078 ± 0.005	0.1
VAT *IL6* (RU) 10^{-4}	9.9 (7.7-20.8)	10.2 (6.8-39.5)	0.8
VAT *PRDM16* (RU)	0.0043 ± 0.002	0.0034 ± 0.001	0.09
VAT *UCP1* (RU) * 10^{-5}	8.5 (4.2-25.2)	5 (3-8.5)	0.08
VAT *DIO2* (RU) * 10^{-4}	2.6 (1.9-4.6)	2.2 (1.6-3.8)	0.8

Table 2. Multivariate regression analyses to predict SAT *PRDM16*, *UCP1* and *DIO2* mRNA levels.

SAT *PRDM16* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Firmicutes RA (%)	0.45	0.01	0.45	0.01	0.43	0.01	0.43	0.01
Age (years)	-0.01	0.9	-	-	-	-	-	-
BMI (kg/m^2)	-	-	-0.09	0.5	-	-	-	-
M (mg/kg/min)	-	-	-	-	0.26	0.1	-	-
HbA_{1c} (%)	-	-	-	-	-	-	-0.06	0.7
Adjusted R^2	0.149 (14.9%)		0.158 (15.8%)		0.189 (18.9%)		0.153 (15.3%)	
P value	0.03		0.03		0.02		0.03	
SAT *PRDM16* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Firmicutes RA (%)	0.43	0.01	0.41	0.02	0.43	0.01	0.46	0.008
Sex	0.27	0.1	-	-	-	-	-	-
Diabetes therapy	-	-	-0.14	0.3	-	-	-	-
Hypertension therapy	-	-	-	-	-0.12	0.4	-	-
Dyslipemia therapy	-	-	-	-	-	-	0.04	0.8
Adjusted R^2	0.230 (23%)		0.172 (17.2%)		0.167 (16.7%)		0.154 (15.4%)	
P value	0.009		0.02		0.02		0.03	
SAT *UCP1* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Firmicutes RA (%)	0.45	0.01	0.37	0.03	0.41	0.03	0.43	0.02
Age (years)	0.05	0.7	-	-	-	-	-	-
BMI (kg/m^2)	-	-	-0.39	0.03	-	-	-	-
M (mg/kg/min)	-	-	-	-	-0.11	0.6	-	-
HbA_{1c} (%)	-	-	-	-	-	-	-0.06	0.7
Adjusted R^2	0.146 (14.6%)		0.295 (29.5%)		0.119 (11.9%)		0.147 (14.7%)	
P value	0.05		0.005		0.08		0.04	
SAT *UCP1* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Firmicutes RA (%)	0.45	0.01	0.49	0.01	0.46	0.01	0.49	0.01
Sex	0.11	0.5	-	-	-	-	-	-
Diabetes therapy	-	-	0.15	0.4	-	-	-	-
Hypertension therapy	-	-	-	-	0.08	0.6	-	-
Dyslipemia therapy	-	-	-	-	-	-	0.15	0.4
Adjusted R^2	0.145 (14.5%)		0.155 (15.5%)		0.139 (13.9%)		0.156 (15.6%)	
P value	0.05		0.04		0.05		0.04	
SAT *DIO2* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**P**
Firmicutes RA (%)	0.39	0.03	0.35	0.06	0.51	0.01	0.40	0.03
Age (years)	-0.36	0.05	-	-	-	-	-	-

BMI (kg/m^2)	-	-	-0.09	0.6	-	-	-	-
M (mg/kg/min)	-	-	-	-	-0.31	0.1	-	-
HbA_{1c} (%)	-	-	-	-	-	-	0.14	0.4
Adjusted R^2	0.201 (20.1%)		0.081 (8.1)		0.222 (22.2%)		0.083 (8.3%)	
P value	0.02		0.16		0.03		0.11	
SAT *DIO2* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Firmicutes RA (%)	0.38	0.04	0.30	0.1	0.27	0.1	0.28	0.1
Sex	-0.26	0.1	-	-	-	-	-	-
Diabetes therapy	-	-	-0.18	0.3	-	-	-	-
Hypertension therapy	-	-	-	-	-0.45	0.01	-	-
Dyslipemia therapy	-	-	-	-	-	-	-0.32	0.09
Adjusted R^2	0.142 (14.2%)		0.097 (9.7%)		0.276 (27.6%)		0.166 (16.6%)	
P value	0.05		0.1		0.008		0.04	

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Table 3. Multivariate regression analyses to investigate the contribution of circulating acetate on SAT *PRDM16* mRNA levels.

SAT *PRDM16* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Plasma acetate (ppm)	0.33	0.07	0.42	0.02	0.42	0.02	0.38	0.04
Ruminococcaceae RA	0.30	0.08	-	-	-	-	-	-
Age (years)	-	-	-0.01	0.9	-	-	-	-
BMI (kg/m^2)	-	-	-	-	-0.02	0.8		
M (mg/kg/min)	-	-	-	-	-	-	0.15	0.5
Adjusted R^2	0.230 (23%)		0.124 (12.4%)		0.124 (12.4%)		0.176 (17.6%)	
P value	0.01		0.05		0.05		0.04	
SAT *PRDM16* (RU)	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Plasma acetate (ppm)	0.39	0.02	0.37	0.04	0.43	0.01	0.42	0.02
Sex	0.26	0.1	-	-	-	-	-	-
Diabetes therapy	-	-	-0.13	0.4	-	-	-	-
Hypertension therapy	-	-	-	-	-0.23	0.1	-	-
Dyslipemia therapy	-	-	-	-	-	-	-0.03	0.8
Adjusted R^2	0.195 (19.5%)		0.152 (15.2%)		0.183 (18.3%)		0.125 (12.5%)	
P value	0.01		0.04		0.02		0.05	

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Figure legends

Figure 1. a-d) Bivariate correlations among *PRDM16* and *UCP1* mRNA levels and firmicutes (a-b) and bacteroidetes (c-d) relative abundance.

Figure 2. a) Circulating acetate levels according to insulin resistance. b-f) Bivariate correlations between circulating acetate and firmicutes relative abundance (b), SAT *PRDM16* mRNA levels (c), insulin sensitivity (d), HOMA-IR (e) and fasting triglycerides (f). g) Relative abundance of families belonging to Firmicutes phylum**.**

Figure 3. HeatMap showing bivariate correlations among the relative abundance of families belonging to Firmicutes phylum and metabolic parameters. *$p<0.05$ and **$p<0.01$.

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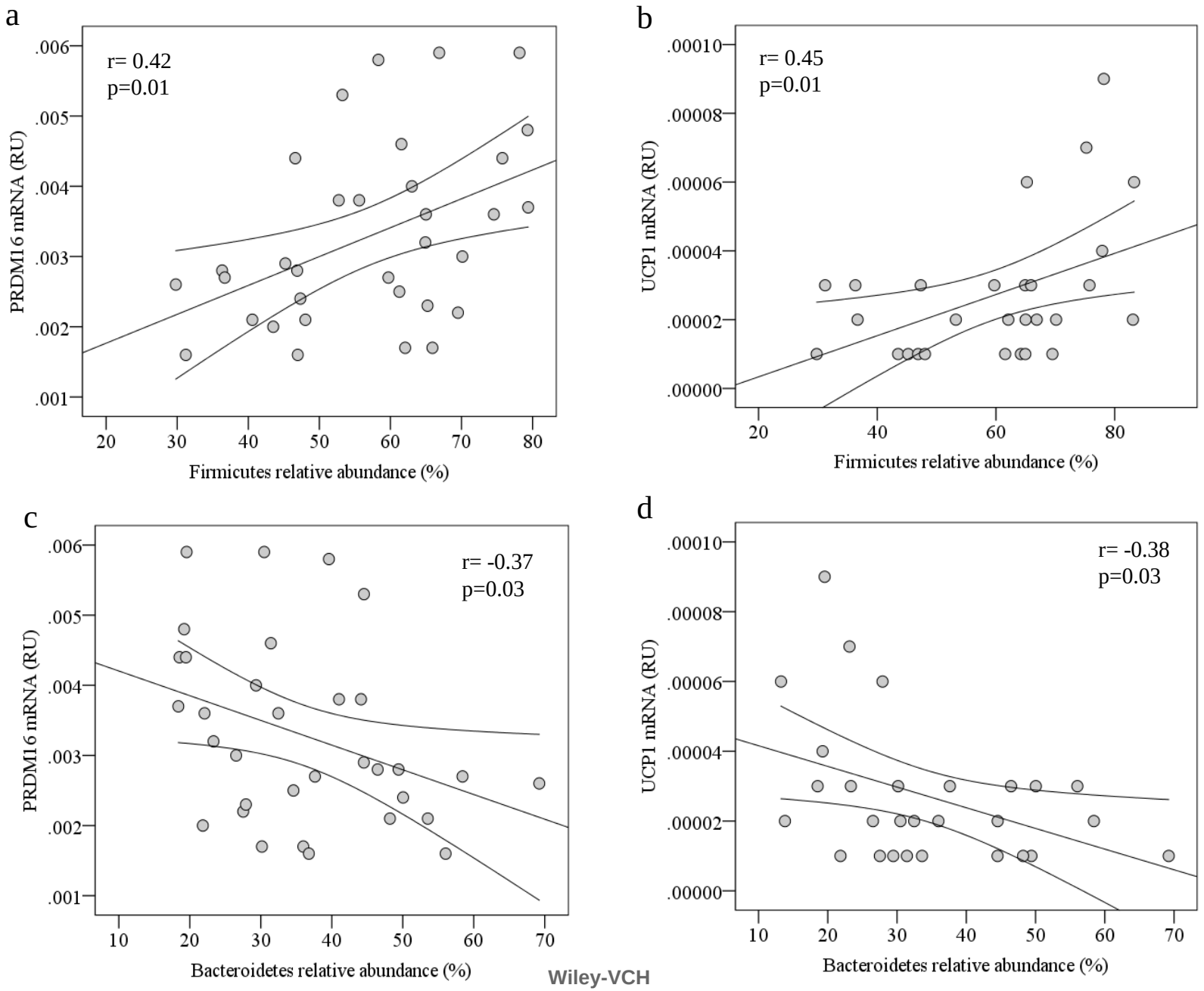
Figure 1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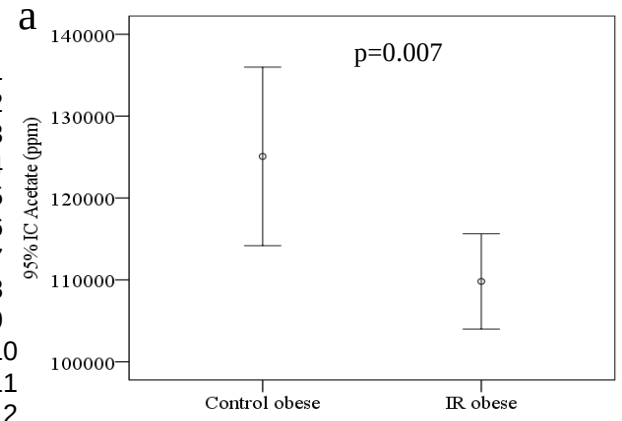
r= 0.42
p=0.01
PRDM16 mRNA (RU)
Firmicutes relative abundance (%)
b
r= 0.45
p=0.01
UCP1 mRNA (RU)
Firmicutes relative abundance (%)
c
r= -0.37
p=0.03
PRDM16 mRNA (RU)
Bacteroidetes relative abundance (%)
d
r= -0.38
p=0.03
UCP1 mRNA (RU)
Bacteroidetes relative abundance (%)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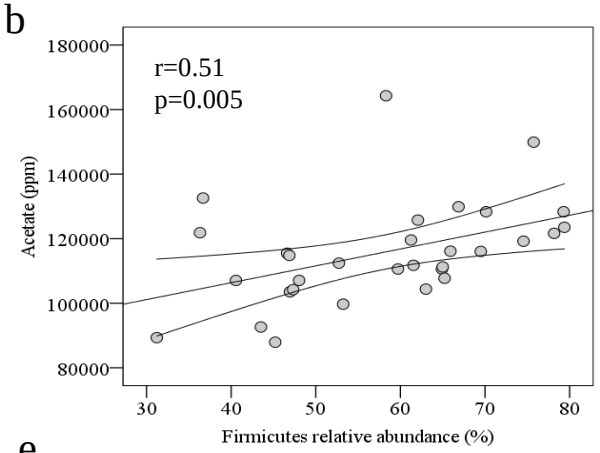
p=0.007
95% IC Acetate (ppm)
140000
130000
120000
110000
100000
Control obese
IR obese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r=0.51
p=0.005
Acetate (ppm)
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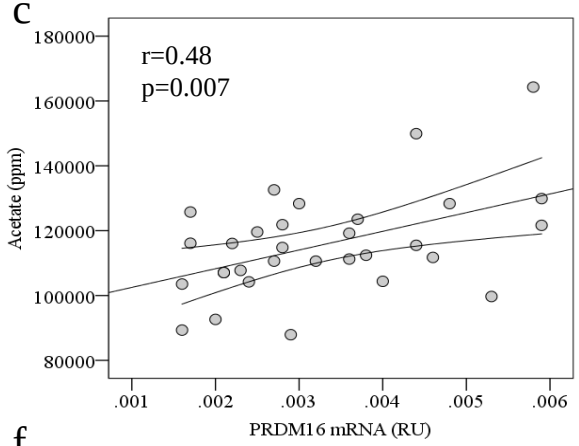
140000
120000
100000
80000
30
40
50
60
70
80
Firmicutes relative abundance (%)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p=0.007
Acetate (ppm)
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140000
120000
100000
80000
.001
.002
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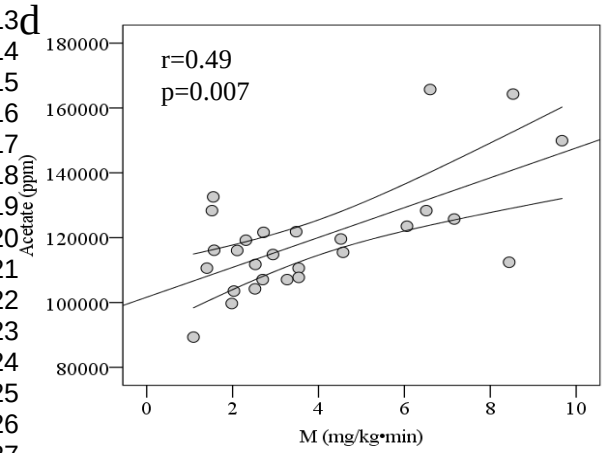
.004
.005
.006
PRDM16 mRNA (RU)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r=0.49
p=0.007
Acetate (ppm)
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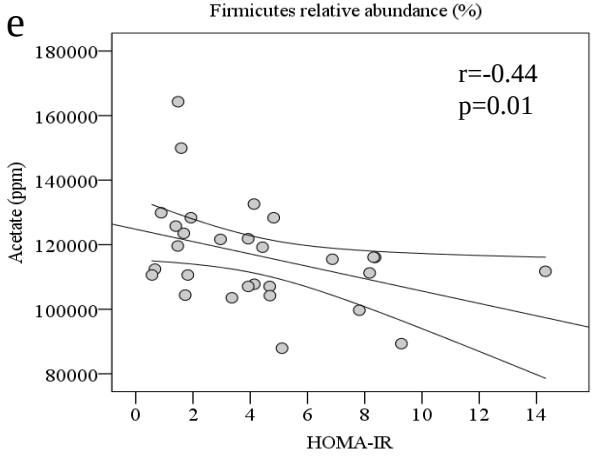
140000
120000
100000
80000
0
2
4
6
8
10
M (mg/kg•min)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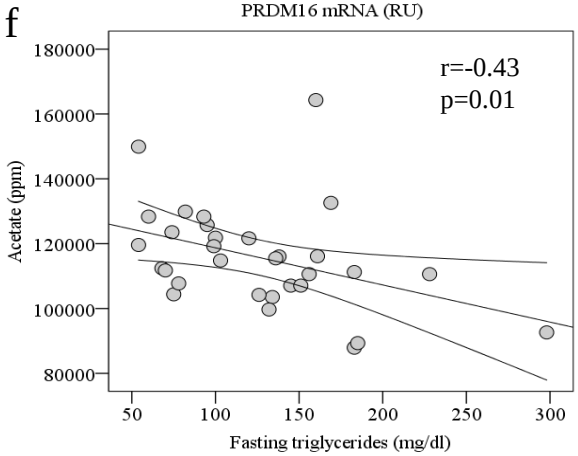
4
6
8
10
12
14
HOMA-IR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160000
140000
120000
100000
80000
50
100
150
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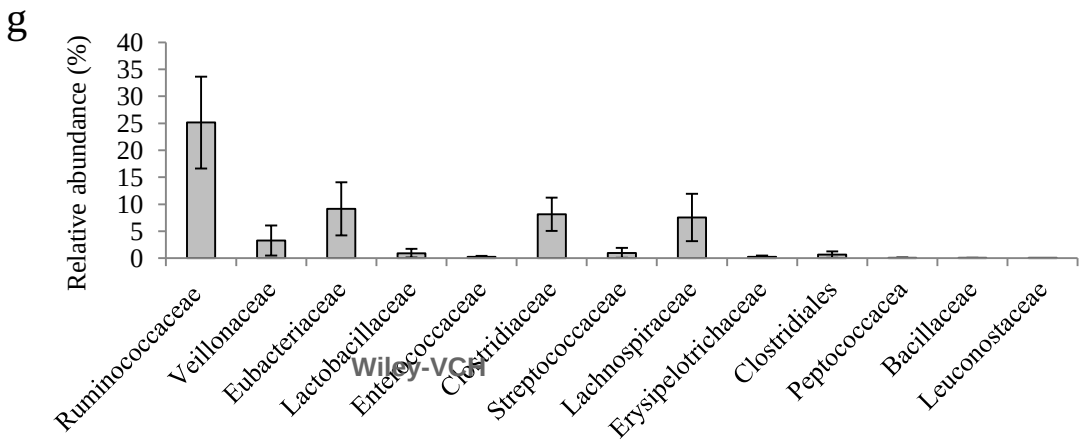
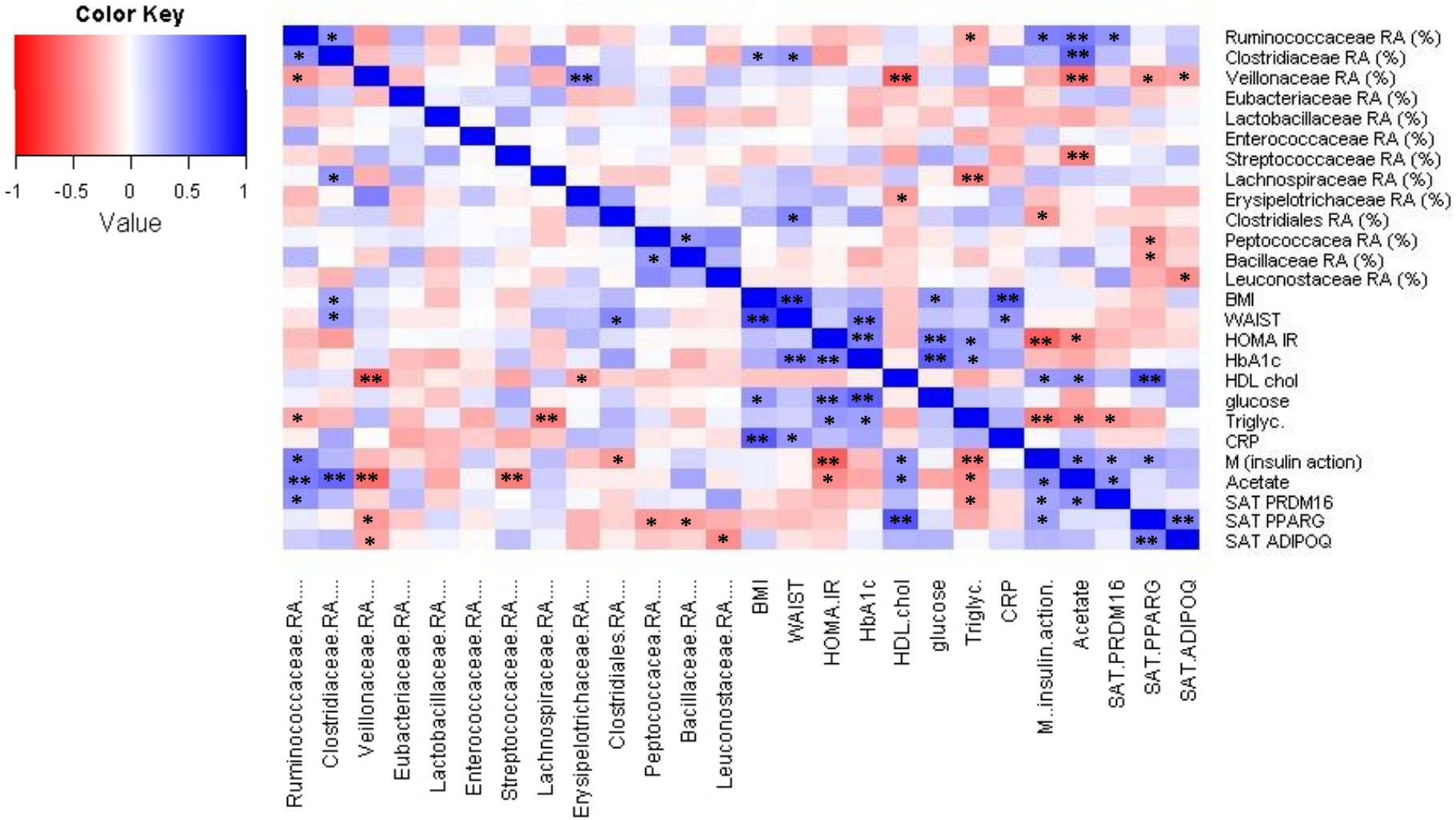
250
300
Fasting triglycerides (mg/dl)


g
Relative abundance (%)
40
35
30
25
20
15
10
5
0
Ruminococcaceae
Veillonaceae
Eubacteriaceae
Lactobacillaceae
Enterococcaceae
Clostridiaceae
Streptococcaceae
Lachnospiraceae
Erysipelotrichaceae
Clostridiales
Peptococcacea
Bacillaceae
Leuconostaceae

Wiley-VCH

Figure 3


Color Key
-1
-0.5
0
0.5
1
Value
Ruminococcaceae RA (%)
Clostridiaceae RA (%)
Veillonaceae RA (%)
Eubacteriaceae RA (%)
Lactobacillaceae RA (%)
Enterococcaceae RA (%)
Streptococcaceae RA (%)
Lachnospiraceae RA (%)
Erysipelotrichaceae RA (%)
Clostridiales RA (%)
Peptococcacea RA (%)
Bacillaceae RA (%)
Leuconostaceae RA (%)
BMI
WAIST
HOMA IR
HbA1c
HDL chol
glucose
Triglyc.
CRP
M (insulin action)
Acetate
SAT PRDM16
SAT PPARG
SAT ADIPOQ
Ruminococcaceae.RA....
Clostridiaceae.RA....
Veillonaceae.RA....
Eubacteriaceae.RA....
Lactobacillaceae.RA....
Enterococcaceae.RA....
Streptococcaceae.RA....
Lachnospiraceae.RA....
Erysipelotrichaceae.RA....
Clostridiales.RA....
Peptococcacea.RA....
Bacillaceae.RA....
Leuconostaceae.RA....
BMI
WAIST
HOMA.IR
HbA1c
HDL.chol
glucose
Triglyc.
CRP
M..insulin.action.
Acetate
SAT.PRDM16
SAT.PPARG
SAT.ADIPOQ

Graphical abstract-text

This figure indicates the association among *Ruminococcaceae* family, plasma acetate levels, SAT expression of browning-related genes and insulin sensitivity, suggesting that increased Ruminococcaceae-enhanced acetate biosynthesis might promote SAT browning and systemic insulin sensitivity in morbidily obese subjects.

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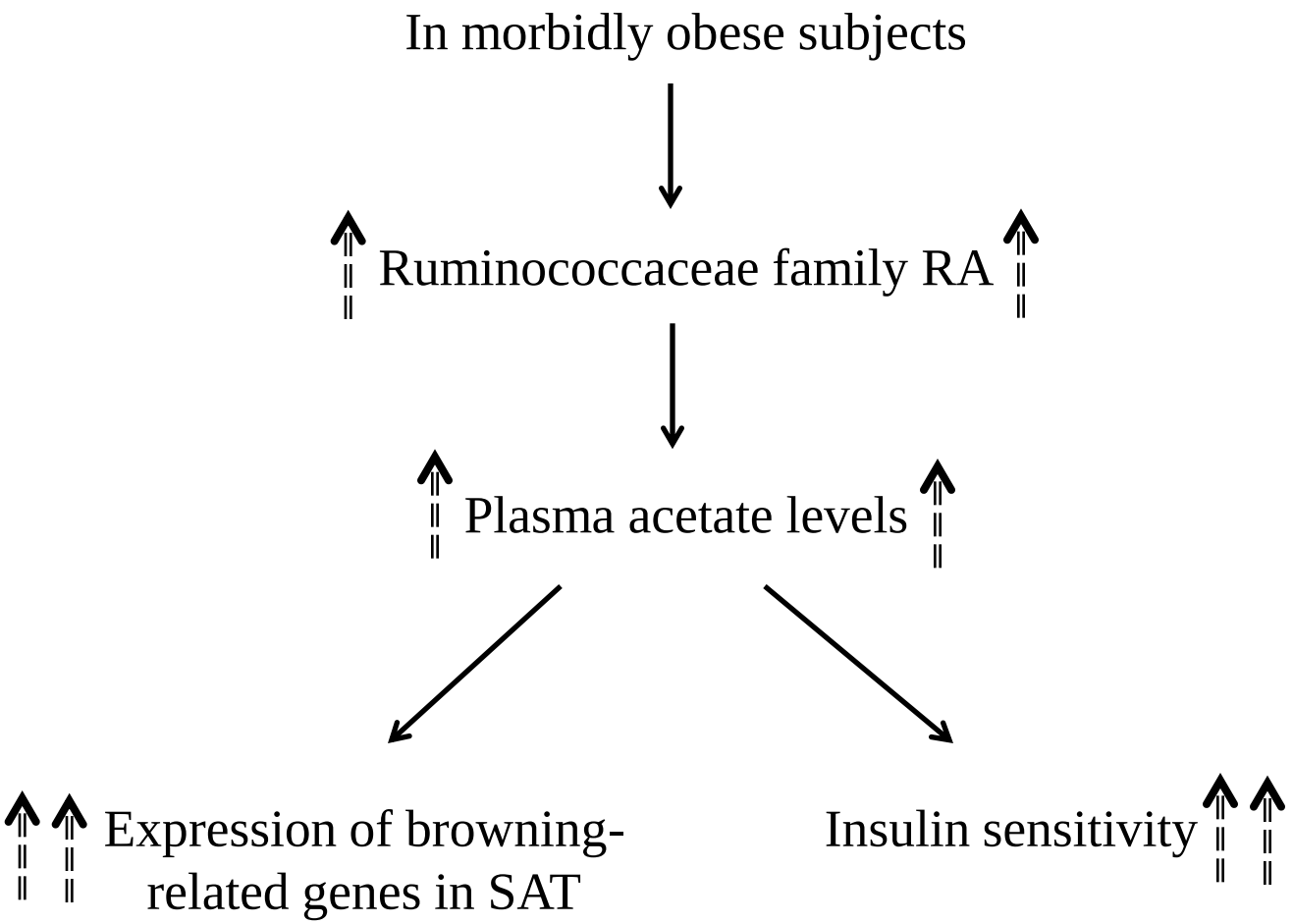
Molecular Nutrition and Food Research

Graphical Abstract