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Botanicals:
A new class of prebiotic agent?
The Rosemary extract example

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Introduction

Since its origin, the concept of "prebiotic" has been revisited several times in the past decade but is still mostly focused on dietary fibres and gastrointestinal events so that the definition recognizes that prebiotics are "selectively fermented ingredients that promote the selective stimulation of growth and/or activities of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host". Plants extracts enriched in bioactive compounds are more and more investigated as a way to fight metabolic disorders and obesity related pathologies, but even if there is growing evidence that specific plants compounds such as polyphenols could modulate gut microbiota, the prebiotic concept is still not consensually expanded to botanicals.

The objective of the study was to determine if the effect of a rosemary (*Rosmarinus officinalis*, Lamiacae) extract (RE) enriched in diterpenoids (mostly carnosic acid and derivatives; 53% w/w) on weight management and metabolic parameters improvement could be attributed to modifications of the microbiota composition and/or activity in a rat model of metabolic disorders and obesity.

Methods

5-weeks old lean (Le) or obese (Ob) female Zucker rats

64 days of treatment:

Lean (fa/+) (n=14)		Obese (fa/fa) (n=10)	
Control diet (CTL) (n=7)	Diet with RE (0.5% w/w) (n=7)	Control diet (CTL) (n=5)	Diet with RE (0.5% w/w) (n=5)

Parameters evaluated at the end and during the treatment:
- Body weight, food and water intake
- Blood triglycerides (TGs), total cholesterol

Parameters evaluated at the end of treatment:
- Inflammatory markers (IL1β, TNFα), GROα chemokine and adipokines (leptin, adiponectin, resistin)
- Small intestine content: α-amylase activity
- Caecum content analysis: bacterial population by qPCR, β-glucosidase activity
- Fecal samples analysis: composition (water, protein, fat, total dietary fiber), short chain fatty acids (SCFA)

Results

The RE reduced body weight gain in both lean and obese counterparts (Figure 1) but reduced serum triglycerides and cholesterol (Figure 2) and significantly decreased circulating TNF-α, IL-1β and leptin and increased adiponectin (Figure 3) only in lean rats, giving evidence of a significant anti-inflammatory effect but only in these animals.

The RE increased the number of *Blautia coccoides* and *Bacteroides/Prevotella* groups and reduced the *Lactobacillus/Leuconostoc/Pediococccus* group in the caecum of lean and obese Zucker rats (Figure 4), which could be correlated to weight loss and low energy harvest. In addition, the intake of the RE reduced caecum β-glucosidase activity (>90%, Figure 5) and increased the excretion of fecal fiber (7–8%, not shown) in the two types of animals that may contribute to reduce dietary energy extraction and to moderate body weight gain. Interestingly, The RE increased the levels of the *Bifidobacterium* genus and reduced those of the *Clostridium leptum* group only in the lean animals (Figure 4) and PCA analysis for bacterial groups and serum variables (Figure 6) revealed that lean rats were able to respond to the RE consumption more flagrantly than obese counterparts, supporting an host genetic mediated effect on the microbiota and on its response to dietary compounds. RE also differentially affected SCFA excreted in the feces of lean and obese rats (Figure 7) reflecting important differences in the production, absorption or metabolism of these compounds and that may partially explain different production and or signaling of inflammatory cytokines, different gut hormones production and different cholesterol/lipids synthesis/metabolism and warrant further research.

Conclusion

Our results show that plant bioactive compounds such as those contained in the rosemary extract (mainly diterpenoids) or their derived metabolites are able to modify gut microbiota composition and to promote beneficial changes with an impact into host metabolism and inflammatory response. Therefore these compounds should be considered as prebiotic or as compounds with "prebiotic-like" effects. Further researches are still required to fully characterize their mechanism of action and to demonstrate an effect in humans.

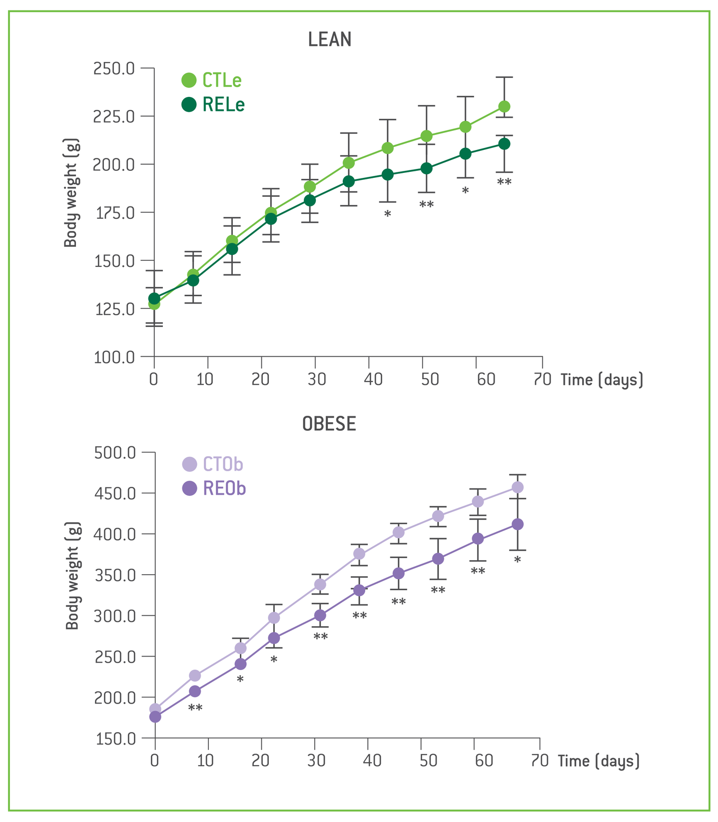

Figure 1: Effects of the consumption of RE on body weight. Data are presented as the mean value ±SD. * p<0.05, ** p<0.01 compared to their respective CT values. (1)

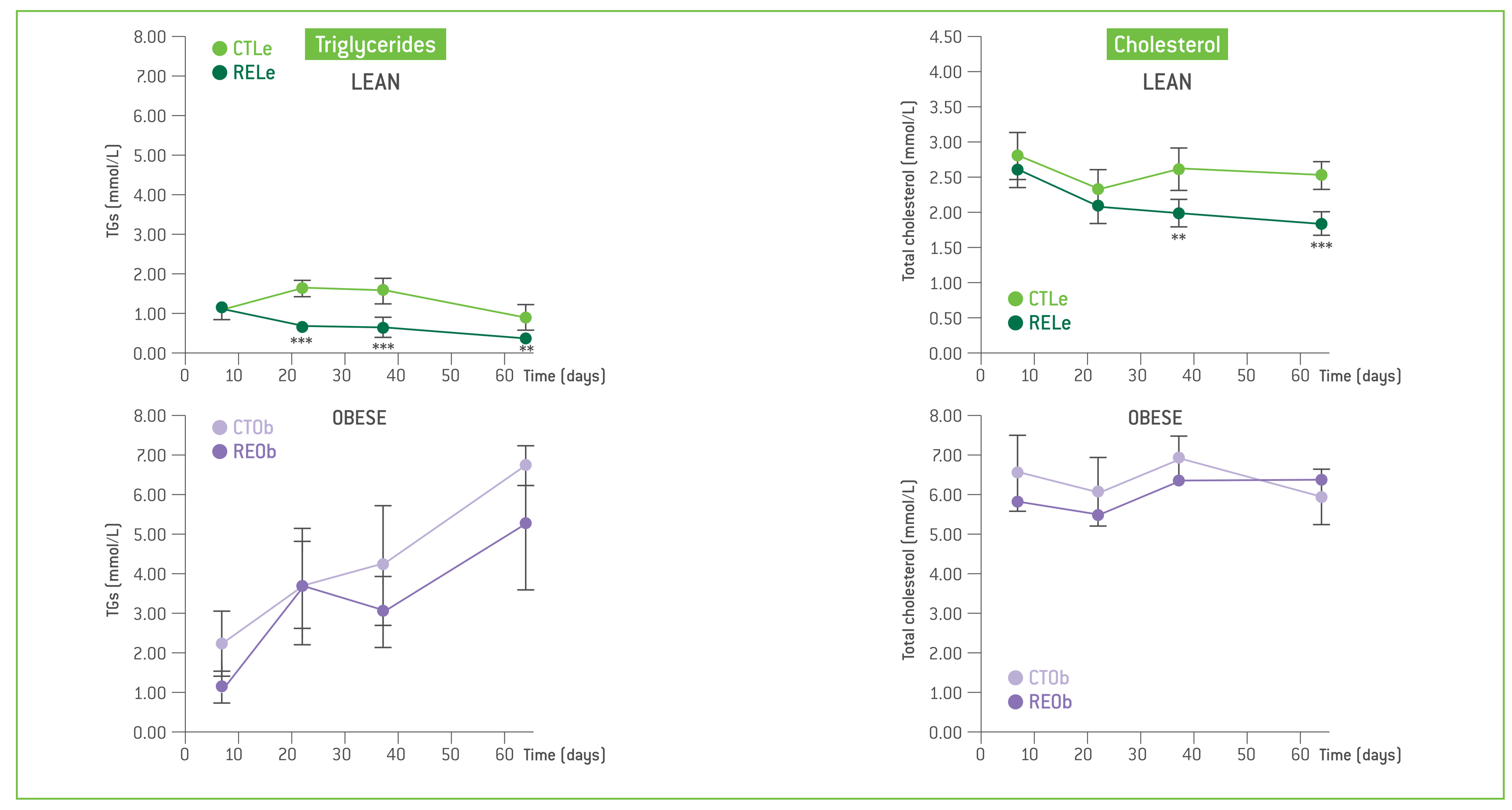

Figure 2: Effects of the consumption of RE on plasma levels of TGs and total cholesterol (mmol/L). Data are presented as the mean value ±SD. ** p<0.01, *** p<0.001 compared to their respective CT values. (1)

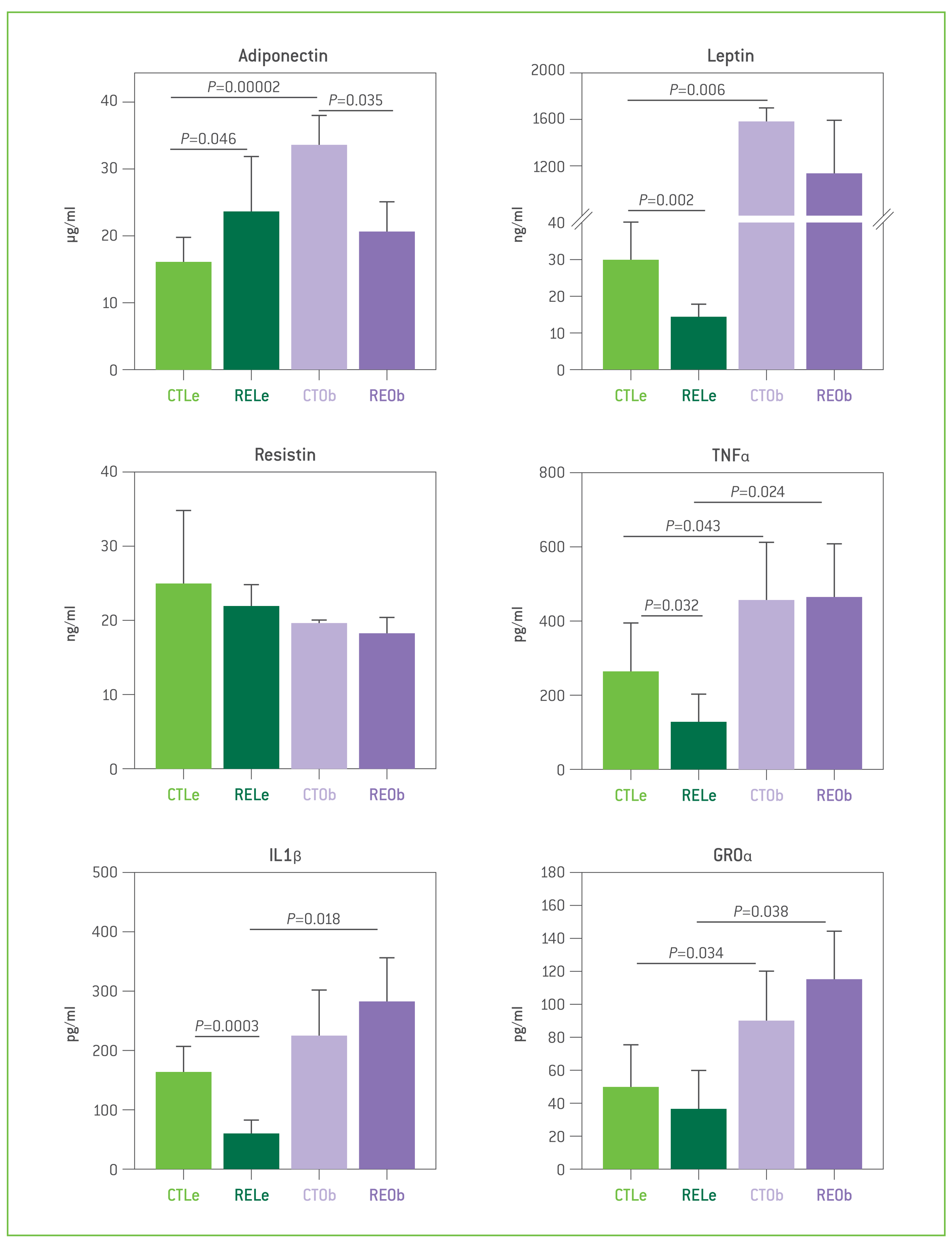

Figure 3: Effects of the consumption of RE on circulating levels of adiponectin, leptin, resistin, TNFα, IL1β and GROα. Data are expressed as means ± SD. Significant p-values (<0.05) between groups are indicated. (2)

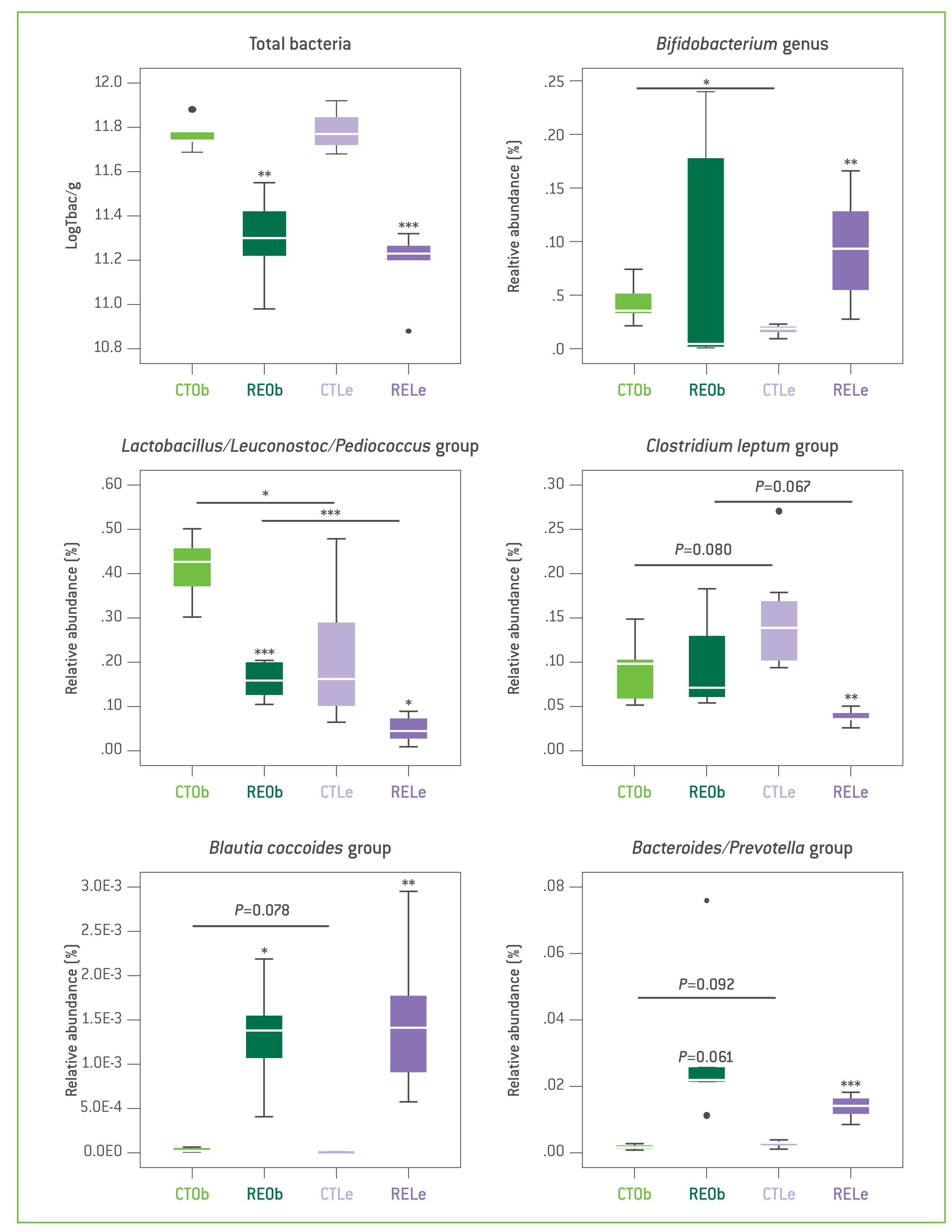

Figure 4: Effects of the consumption of RE on the different bacterial populations in the caecum. Data are presented as box-and-whisker plots and as log10 of the value per gram of caecum content (fresh weight) for Total bacteria (Tbac) and as relative abundance (%) of total bacteria for each group. Horizontal lines represent the comparison between obese and lean rats. Significant differences are indicated by *p<0.05, **p<0.01, ***p<0.001. p values below 0.1 are also displayed as indication of a trend.

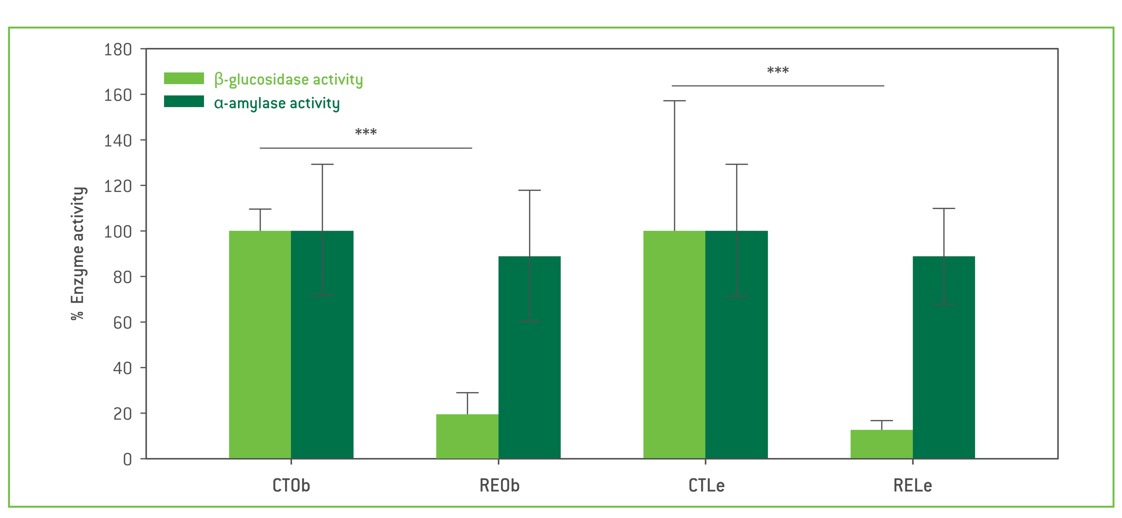

Figure 5: Effects of the consumption of RE on α-amylase activity in the small intestine and β-glucosidase activity in the caecum. Results are shown as % of inhibition respect to the activity in the corresponding control (CT) group. Significant differences are indicated by ****p<0.001.

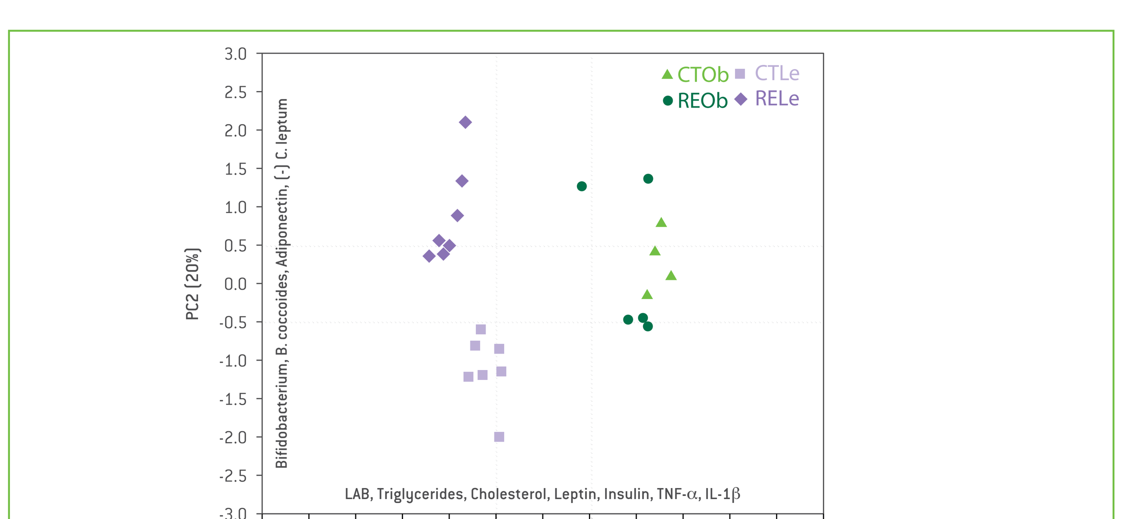

Figure 6: Discriminant principal component analysis (PCA). Analysis of the caecum bacterial groups in obese (Ob) and lean (Le) rats and the serum triglycerides, cholesterol, leptin, insulin, adiponectin, TNFα, and IL1β. The 2 components accounted for 67% of the total variability. PC1 and PC2 clearly differentiated between the lean and the obese phenotypes (p<0.001). PC1 and PC2 also discriminated between control and RE-supplemented lean rats (P<0.001) but not between control and supplemented obese rats.

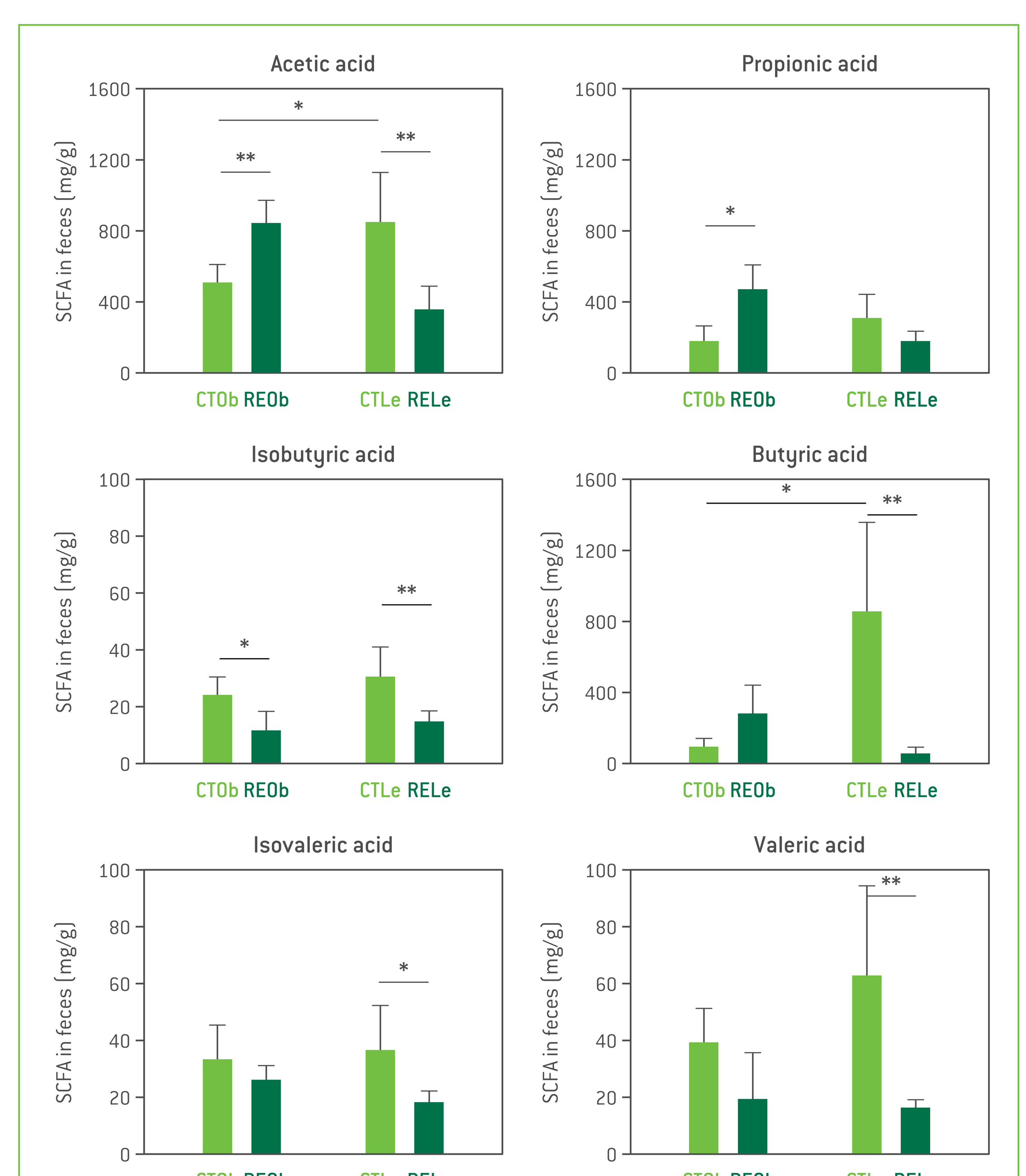

Figure 7: Effects of the consumption of RE on SCFA fecal composition. Results are shown as µg/g of feces (fresh weight). Significant differences between groups are indicated by *p<0.05, **p<0.01.

References

(1) Romo-Vaquero *et al.*, 2012. Inhibition of Gastric Lipase as a Mechanism for Body Weight and Plasma Lipids Reduction in Zucker Rats Fed a Rosemary Extract Rich in Carnosic Acid. *Plos* One, 7 (6): e39773.
(2) Romo-Vaquero *et al.*, 2013. A rosemary extract enriched in carnosic acid improves circulating adipocytokines and modulates key metabolic sensors in lean Zucker rats: Critical and contrasting differences in the obese genotype. *Mol. Nutr. Food Res.*, 58: 942-53.
(3) Romo-Vaquero *et al.*, 2014. A Rosemary Extract Rich in Carnosic Acid Selectively Modulates Caecum Microbiota and Inhibits β-Glucosidase Activity, Altering Fiber and Short Chain Fatty Acids Fecal Excretion in Lean and Obese Female Rats. Plos One, 9 (4): e94687.

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