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Mediterranean Tomato-Based *Sofrito* Sauce Improves Fibroblast Growth Factor 21 (FGF21) Signaling in White Adipose Tissue of Obese ZUCKER Rats

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Scope: Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Since FGF21 production and signaling are regulated by some bioactive dietary compounds, we analyze the impact of Mediterranean tomato-based *sofrito* sauce on: (i) the FGF21 expression and signaling in visceral white adipose tissue (vWAT), and (ii) the insulin sensitivity of obese Zucker rats (OZR).
Methods and results: OZR are fed with a *sofrito*-supplemented diet or control diet. Insulin sensitivity and FGF21 signaling are determined. We observed that *sofrito* is able to improve the responsiveness to both hormones in obese rats. *Sofrito*-supplemented diet increases FGF21 signaling in vWAT by inducing the expression of the FGF receptors (FGFR1 and FGFR4) that promotes the expression of canonical target genes, like Egr-1, c-Fos and uncoupling protein 1 (Ucp1).
Conclusions: A *sofrito*-supplemented diet improves insulin and FGF21 sensitivity in OZR, explaining part of *sofrito*'s healthy effects on glucose metabolism. In addition, induction of UCP1 and the unchanged body weight despite the hyperphagic behavior of the *sofrito*-fed rats suggests that the increase in FGF21 signaling correlates with an increase in energy expenditure (EE). Further studies in humans may help to understand whether *sofrito* consumption increases the EE in obese individuals.

1. Introduction

Fibroblast Growth Factor 21 (FGF21) or derivatives are promising therapeutic agents for the treatment of metabolic diseases such as obesity, type 2 diabetes, or metabolic syndrome due to its beneficial effects on glucose/lipid homeostasis and body weight control by increasing energy expenditure (EE) and inducing, at least in part, browning and uncoupling protein 1 (UCP1) overexpression in adipose tissues.[1–4] Pharmacological infusion of FGF21 in different genetic or diet-induced animal models of obesity or diabetes causes an improvement in insulin sensitivity, a reduction in glucose and serum lipids levels, and weight loss.[5–9]

FGF21 shows endocrine, paracrine, and autocrine properties and its target tissues are essentially white and brown adipose tissue (WAT, BAT), skeletal muscle, heart, and brain. FGF21 is predominantly produced by the liver but also by other tissues, such as WAT, BAT, skeletal muscle, and pancreas[10–13] in response to different stimuli such as cold[14] and different nutritional challenges.[15] The effects of FGF21 on its target tissues occur through the fibroblast growth factor receptor (FGFR) together with the co-receptor β-klotho (KLB).[16,17] It has been proposed that obesity is an FGF21-resistant state due to a downregulated expression of FGFRs and KLB that impairs FGF21 signaling.[18,19] Most of the data show

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that pharmacological administration of FGF21 has beneficial metabolic effects; a remaining question is whether it is possible to restore the endogenous FGF21 signaling in obese people to improve their metabolic parameters and reduce the risk of developing obesity-associated pathologies.

The Mediterranean diet as part of a lifestyle is considered a model of healthy eating, with protective properties in terms of morbidity and mortality from different metabolic diseases.[20–22] The health-promoting effects of this diet have been in part attributed to a high intake of bioactive dietary compounds, such as polyphenols and carotenoids present in high amounts in fruits, vegetables, cocoa, and some beverages such as tea, coffee, or wine. Various epidemiological studies show that their regular consumption is associated with many beneficial effects on metabolic diseases.[22,23] Several lines of evidence support the notion that polyphenol-rich diets play a key role in regulating lipid and glucose metabolism and are thus pivotal in the prevention and treatment of pathologies related to energy homeostasis.[24–29] Besides polyphenols, consumption of lycopene, a bioactive compound that belongs to the carotenoid family, or tomato extracts as a lycopene source, has been related to healthy effects in cardiovascular diseases (CVD), diet-induced obesity (DIO), metabolic disorders, inflammation, and adiposity.[30]

Within the Mediterranean diet, tomato and tomato sauces are typical and *sofrito* is one of the most consumed. *Sofrito* is a cooked sauce made with tomato, onion, olive oil, and in some cases garlic, which, due to its composition and cooking method (mechanical and thermal treatments), has a high bioavailable content of diverse bioactive compounds that includes carotenoids such as lycopene and polyphenols belonging to different chemical groups.[31–37]

In terms of the healthy properties of bioactive dietary compounds, one of the most important questions concerns the molecular mechanism by which they exert their beneficial effects. It has been recently described that in various rodent models of DIO there exists a positive correlation between the beneficial effects of polyphenol-rich fruit extracts and FGF21 activity due to an induction of FGF21 levels[38,39] or an improvement of the FGF21 signaling by increasing the expression of FGF21 receptors and KLB.[40,41] Taking into account *sofrito*'s rich bioactive compound content, here we analyzed the impact of a regular consumption of *sofrito* on insulin sensitivity in an obese rat model and identified FGF21 signaling as a mechanism by which *sofrito* could exert, at least in part, its healthy effects. This work is closely related to a previous one where the authors described that *sofrito* protects against vascular alterations that could precede major cardiometabolic complications in obesity.[42]

2. Experimental Section

2.1. Animals and Diets

The animal procedures used in this article were previously described in Rodríguez-Rodríguez et al.[42] Briefly, 8-week-old male obese Zucker rats (OZR) were randomly divided into two groups: (1) OZR fed a chow diet (CD) ($n = 7$); and (2) OZR fed a *sofrito*-supplemented diet (CD with *sofrito* 2% (w/w) ($n = 8$)). We used 8-week-old male lean Zucker rats (LZR) fed a CD as initial control ($n = 7$). Chow diet (Teklad Global 2018) was provided by Harlan Laboratories (Milan, Italy) and *sofrito* by Gallina Blanca-Star (Barcelona, Spain). *Sofrito's* nutritional composition is described in Supporting Information Table S1. The percentage of *sofrito* supplementation (2%) was calculated considering a human consumption of one serving of *sofrito* per day with the meals and the lycopene content in liver was used as a biomarker of *sofrito* intake.

Body weight and food intake were evaluated weekly. After 8 weeks of nutritional intervention, animals were sacrificed and blood samples, liver, and visceral adipose tissue (vWAT: perirenal and retroperitoneal) were collected. The protocol for animal handling and experimentation was approved by the Committee of Ethical Experimentation of the University of Barcelona (557/16).

2.2. Serum Measurements

FGF21 in serum samples was measured with a mouse/rat FGF21 ELISA (ref. EZRMFGF21-26K) obtained from EMD Millipore (Germany). The assay was conducted following the manufacturer's protocol. Briefly, a calibration curve was constructed by plotting the difference in absorbance values at 450 and 590 nm versus the FGF21 concentrations of the calibrators. Concentrations of unknown samples (performed in duplicate) were determined using this calibration curve. The Millipore's protocol indicates that the lower limit of sensitivity is 10.0 pg mL^{-1} and the measurement error in the range of concentration of our samples is 6%.

2.3. Insulin Tolerance Test

Before the end of the treatment, insulin tolerance test (ITT) was performed on rats after fasting for 3 h. Blood samples were collected from the tail vein before and after 30, 60, and 120 min of insulin intraperitoneal administration (0.75 U kg^{-1} insulin solution, Sigma–Aldrich Chemical Co, USA). Plasma glucose concentration was determined using a blood glucose commercial monitoring meter (Contour NEXT, Bayer, Spain). The area under the glucose curve (AUC) was calculated using Prism GraphPad 5.01 software.

2.4. RNA Isolation and Relative Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from the frozen tissues using total RNA isolation (TRI) reagent solution (AM9738, ThermoFisher Scientific, USA) followed by DNase I treatment (AM1906, ThermoFisher Scientific, USA). To measure the relative mRNA levels, qRT-PCR was performed using SYBR Green or TaqMan probes. cDNA was synthesized by MMLV reverse transcriptase (28025021, ThermoFisher Scientific, USA) with random hexamers (11034731001, Roche Diagnostics, Germany). The TaqMan Gene Expression Master Mix (4369514) and SYBR Green PCR Master Mix (4364344), supplied by ThermoFisher Scientific (USA), were used for the PCR step. Amplification and detection were performed using the BioRad CFX96 touch (BioRad, USA). 18S and beta-actin were used to normalize the mRNA levels. The

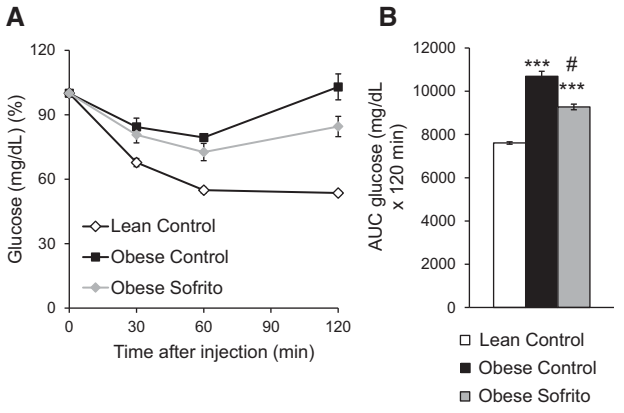

Figure 1. Insulin resistance is attenuated by *sofrito*-supplemented diet in OZR. A) ITT showing plasma glucose after intraperitoneal administration of insulin (0.75 U kg^{-1}) in lean control rats, obese control rats, and obese *sofrito*-supplemented rats after 7 weeks of diet administration. B) AUC of glucose levels. Data are presented as the mean ± SEM. ***$p < 0.001$ versus lean control rats; #$p < 0.05$ versus obese control rats. (n = 6–8/group).

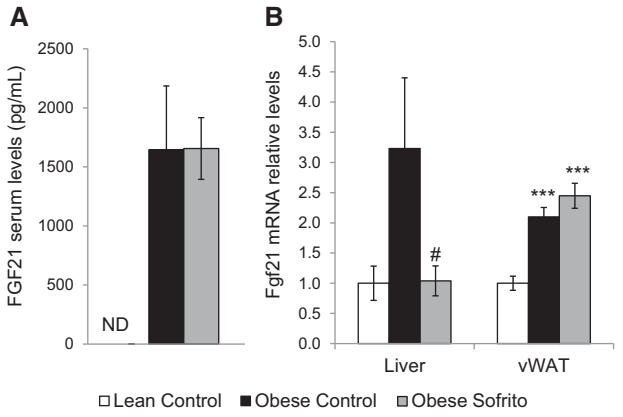

Figure 2. FGF21 serum levels are increased in obese phenotype due to higher expression in liver and vWAT but not influenced by *sofrito*. A) FGF21 protein levels (pg/mL) were measured by ELISA in plasma of lean control rats, obese control rats, and obese *sofrito*-supplemented rats. B) The mRNA relative levels of *Fgf21* were measured by qRT-PCR in liver and vWAT of lean control rats, obese control rats and obese *sofrito*-supplemented rats. Data are presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus lean control rats; #$p < 0.05$ versus obese control rats (n = 6–8/group).

primer sequences or Taqman Probes used are shown in supplemental information (Supporting Information Table S2). Results were obtained by the relative standard curve method and expressed as fold increase compared to the experimental control.

2.5. Data Analysis/Statistics

All data are expressed as mean ± SEM. The gene expression assays are expressed as mRNA relative levels and referred to 1 assigned to LZR control or OZR control, as indicated. Significant differences were assessed by a two-tailed Student's *t*-test.

3. Results and Discussion

3.1. Insulin Resistance is Attenuated by *Sofrito* Supplemented-Diet in OZR

Obesity is usually associated with insulin resistance and glucose intolerance. OZR showed increased levels of blood glucose and those values are not significantly changed by a CD supplemented with *sofrito*.[42] To test the effect of *sofrito* on insulin sensitivity we performed an ITT with the different experimental groups. Both groups of OZR showed insulin resistance as demonstrated by a significant increase in the AUC (**Figure 1**A) compared to LZR, whereas the *sofrito*-supplemented diet was capable of partially counteracting this insulin resistance in OZR (Figure 1B).

3.2. FGF21 Serum Levels are not Influenced by *Sofrito*

Taking into account that FGF21 and bioactive dietary compounds are both described as beneficial signals for insulin sensitivity, we decided to evaluate the role of FGF21 expression and signaling on the insulin sensitizing effect of *sofrito* on OZR. We analyzed the FGF21 protein levels in serum by an ELISA assay. The serum levels of FGF21 are higher in both groups of OZR than the corresponding littermates (LZR) (**Figure 2**A). These results are in accordance with previously published studies that have shown increased FGF21 serum levels compared with normal weight littermates in several models of obesity.[18,43,44]

In this case, the FGF21 levels detected in OZR fed the *sofrito*-supplemented diet were almost the same as those in the obese control rats, indicating that *sofrito* is not able to modify the circulating levels of FGF21. It is worth mentioning that the circulating levels of FGF21 in healthy individuals (LZR) were undetectable. These results reinforce the idea that FGF21 production is directly related to metabolic stress conditions such as obesity.

To determine the source of FGF21 in OZR we determined the expression levels of FGF21 in the liver and vWAT as one of the main target tissues of obesity. Figure 2B shows the relative mRNA levels of FGF21 in the liver and vWAT. Contrasting with the unchanged circulating levels of FGF21, OZR fed with the *sofrito*-supplemented diet showed a significant reduction in the mRNA levels of FGF21 in the liver. In vWAT we observed that in both OZR groups the relative mRNA levels of FGF21 were twofold higher than in LZR. These results highlight that an extra hepatic production of FGF21 could be, at least in part, responsible of the higher FGF21 circulating levels detected in OZR. Tissue-specific knockout models would be necessary to elucidate the source of FGF21 under different nutritional conditions.

Since the production of FGF21 is not modified by *sofrito*, we studied the FGF21 signaling as the presumed molecular pathway responsible for the health benefits of *sofrito* on glucose metabolism. As the effects of *sofrito* on insulin sensitivity were observed only in OZR, the next experiments were performed only with those rats.

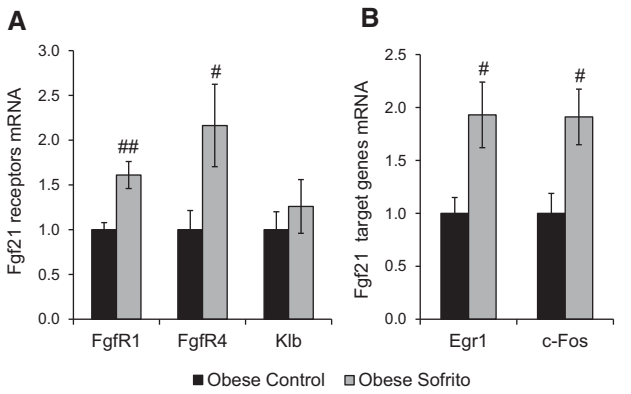

Figure 3. FGF21 signaling is improved in vWAT of OZR fed with a *sofrito*-supplemented diet. The mRNA relative levels of *Fgf21* receptors (*Fgfr1*, *Fgfr4*, *Klb*) A) and the mRNA relative levels of the target genes of the FGF21 signal transduction pathway (*Egr1* and *c-Fos*) B) were measured by qRT-PCR in vWAT of obese control rats and obese *sofrito*-supplemented rats. Data are presented as the mean ± SEM. #$p < 0.05$ ##$p < 0.01$ versus obese control rats. ($n = 6$–8/group).

3.3. FGF21 Signaling is Improved in vWAT of OZR Fed with a *Sofrito*-Supplemented Diet

FGF21 signaling requires the dimerization of the FGFR and KLB and the sensitivity of a tissue or organ to FGF21 is directly dependent on the coexpression of both receptors. In DIO mice, the FGF21-resistant state implies an attenuated responsiveness in the liver and WAT as a result of a reduction in FGFR1, FGFR4, and/or KLB levels.[18,45,46] We tested the FGFR1, FGFR4, and KLB expression in the liver and vWAT. **Figure 3**A shows that the expression of FGFR1 and FGFR4 were significantly increased in rats fed with the *sofrito*-supplemented diet compared to obese control rats, and although KLB expression did not reach statistical significance it shows a tendency to increase. The mRNA levels of the receptors in the liver were unchanged by *sofrito* (data not shown).

Taken as a whole, our data led us to hypothesize that *sofrito* restores the FGF21 signaling in OZR, overcoming the FGF21-resistant state characteristic of obesity. These results were in accordance with previously published data demonstrating that polyphenols or polyphenols-enriched foods are able to increase the expression of FGF21 receptors.[40,41]

To confirm the impact of *sofrito* on the FGF21 signaling pathway we analyzed the mRNA levels of the FGF21 target genes c-Fos and Egr-1 in vWAT. The expression of c-Fos and Egr-1 were significantly induced in rats fed with the *sofrito*-supplemented diet compared to the obese control rats (Figure 3B). This induction represents a direct measurement of FGF21 signaling and tissue responsiveness and led us to confirm the capacity of *sofrito* to improve the sensitivity of vWAT to FGF21 in OZR.

3.4. *Sofrito* Induces UCP1 Expression in the vWAT of OZR

During the nutritional intervention with *sofrito*, the body weight and food intake of the rats were recorded. As previously described, the caloric intake (Kcal d^{-1} per rat) and body weight of OZR were higher than in LZR (Supporting Information Figure S1); curiously, while the caloric intake was also significantly increased by the inclusion of *sofrito* in OZR's diet (Supporting Information Figure S1A), there were no changes in their body weight (Supporting Information Figure S1B). Looking for an explanation for the above-mentioned profile of OZR fed with *sofrito*-supplemented diet compared to control OZR we hypothesized that the differences could be due to changes in EE. It is well-known that FGF21 administration leads to a reduction in body weight through increased EE.[47] Concretely, the injection of FGF21 increases thermogenic capacity by stimulating the expression of UCP1 and type 2 iodothyronine deiodinase protein 2 (DIO2) in BAT, and UCP1 in WAT, where it produces the so-called browning process.[48,49] Because UCP1, an FGF21 target gene[3,4] has been linked to EE, we analyzed the UCP1 expression in the vWAT collected from OZR. The mRNA levels of UCP1 are increased in the vWAT of *sofrito*-supplemented rats compared to control rats (**Figure 4**), suggesting an effect of *sofrito* on the induction of browning. The process of browning causes the change of white adipocytes to beige/brite adipocytes, which are greater energy consumers, and this could explain the unchanged body weight of the hyperphagic OZR fed the *sofrito*-supplemented diet despite they were consuming more calories. We also analyzed pRDM16, PGC1b, and PPARg expression levels but no significant changes were observed.

Our data indicate that *sofrito* modulates FGF21 signaling without modification of FGF21 expression. The effect of *sofrito* on Egr-1, c-fos, and UCP1 expression indicate that FGFR expression renders a more active signaling receptor complex. Recently, it has been described the role of miRNA34a in downregulation of FGFR1 and KLB levels in adipocytes.[50] However, there are no differences in the miRNA34a expression between OZR and OZR rats fed with *sofrito*-supplemented diet (data not shown). As *Sofrito* is a mix of different bioactive compounds, including polyphenols and carotenoids but also oleic acid (MUFA) from

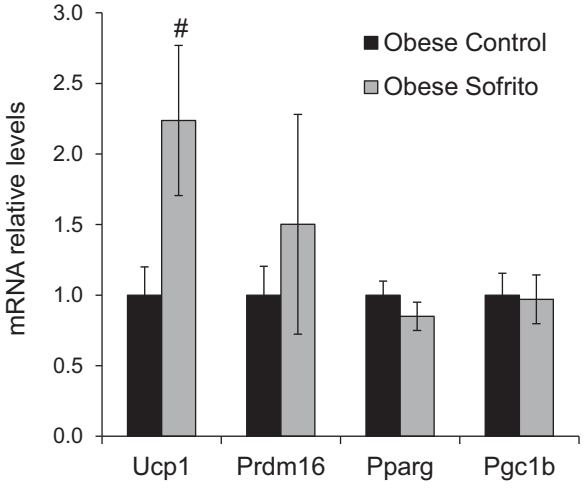

Figure 4. OZR fed with a *sofrito*-supplemented diet show higher UCP1 expression in vWAT. The mRNA relative levels of *Ucp1*, *Prmd16*, *Pparg*, and *Pgc1b* were measured by qRT-PCR in vWAT of obese control rats and obese *sofrito*-supplemented rats. The data are presented in absolute values as the mean ± SEM after 8 weeks of nutritional intervention. #$p < 0.05$ versus obese control rats ($n = 6$–8/group).

olive oil and a cooking processes that modify some molecules bioavailability,[51] further studies are necessary to determine the molecular mechanisms that produce the metabolic response observed, the role of each component or the necessity of the complete combination in the effects of *sofrito* on FGF21 signaling.

4. Concluding Remarks

The Mediterranean diet is considered a healthy eating pattern but in order to convince society of its benefits, and to make its implementation as a lifestyle easier, it is important to have all the information and this includes the molecular mechanisms that can explain its beneficial effects. *Sofrito* is included in many of the typical Mediterranean dishes and every day brings further evidence of its healthy properties. This manuscript discloses one of the putative mechanisms of *sofrito*'s action specifically the one that could explain the improvement in glucose metabolism described by some bioactive dietary compounds and also reinforce the healthy properties of *sofrito*. **Translation to humans**: we designed our experimental approach using a dose of *sofrito* that may be close to the dose ingested in humans. The percentage of *sofrito* used represents the intake of one serving of *sofrito* per day in humans **(**https://ndb.nal.usda.gov/ndb/search/list). It is worth to mention that in the Predimed study the intake of two or more servings of *sofrito* per week is considered an indicator of a good adherence to Mediterranean Diet.[52]

Abbreviations

AUC, area under the curve; BAT, brown adipose tissue; CD, chow diet; DIO, diet-induced obesity; Dio2, type 2 iodothyronine deiodinase protein 2; EE, energy expenditure; FGF21, fibroblast growth factor 21; FGFR, fibroblast growth factor receptor; HFD, high-fat diet; ITT, insulin tolerance test; KLB, β-klotho receptor; LZR, lean Zucker rats; OZR, obese Zucker rats; PGC1b, peroxisome proliferator-activated receptor gamma coactivator 1-beta; PPAR, peroxisome proliferator-activated receptor; PRDM16, PR domain containing 16; UCP1, uncoupling protein 1; WAT, white adipose tissue

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgments

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V.S. performed the metabolic characterization of the rats, compiled the statistics, and analyzed the results. R.R. designed and carried out the nutritional intervention and the ITT. U.M. performed the assays suggested by the reviewers. C.R. participated in the experimental approaches. R.M. designed the nutritional intervention and discussed the results. P.F.M., D.H., and J.R. designed the experimental approach to characterize the molecular effects of *sofrito*, supervised the study, discussed the results, and wrote the paper. All authors read, approved, and contributed to the final version of the manuscript.

Conflict of Interest

The authors have declared no conflicts of interest.

Keywords

adipose tissue, fibroblast growth factor 21, insulin resistance, Mediterranean diet, uncoupling protein 1

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