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Proceeding Paper

Exploring Peruvian cocoa populations and their influence on rat metabolism†

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Abstract: This study aimed to characterize two Peruvian cocoa populations (CCN51 and JL2) and to determine their influence on rat metabolism. For this, phytochemical composition and in vitro antioxidant activity was established for CCN51 and JL2. On the other hand, Wistar rats were fed with either CCN51-, JL2-enriched or standard diets for 4 weeks. At the end, an oral glucose tolerance test was carried out. Moreover, visceral adiposity index and fecal pH were measured. The JL2 cocoa showed the highest content of total polyphenols, catechin and epigallocatechin-3-gallate and also the highest in vitro antioxidant activity. No significant differences were observed in glycemia among the three experimental groups, but both cocoa-fed groups showed lower visceral adiposity than the standard diet-fed animals. Lower fecal pH was observed after both cocoa diets. In conclusion, two Peruvian cocoa populations with different polyphenol composition induced similar effects on rat metabolism when administered as 10% of the diet for 4 weeks.

Keywords: *Theobroma cacao*; polyphenols; metabolism

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease that represents a significant public health problem due to its rising prevalence and incidence [1]. The current therapeutic approach for its treatment relies on pharmaceuticals, but lifestyle changes (diet and physical exercise) are the most promising strategies for preventing or delaying the onset of T2DM. Consequently, the identification of dietary components with potential antidiabetic effects has become essential in the search for alternative or adjunct treatments for this disease [2]. In this context, flavonoids are gaining interest for their potential metabolism benefits [3].

2. Material and Methods

2.1. Cocoa Population Characterization

Two cocoa pastes made with beans from the "Chuncho" Peruvian cocoa populations from the Cusco region were used: JL2 and VRAE99. In addition, the CCN51 ordinary cocoa paste from the same area was used as reference.

Polyphenol quantification was carried out by high-performance liquid chromatography with diode-array detection (HPLC-DAD) and the *in vitro* antioxidant capacity was established by the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay. Determinations were performed in triplicate, began with 100% pure cocoa paste.

2.2. In vivo Study

According to their polyphenol content and antioxidant activity, the JL2 cocoa population was selected to evaluate its effects on rat metabolism in an *in vivo* study. The ordinary CCN51 cocoa paste was also included to be considered as a reference cocoa.

2.2.1. Diets and Animals

Three different diets were used: a standard diet based on AIN-93M diet (Envigo, Indianapolis, IN, US) and two diets in which 90% of powdered AIN-93M was mixed with 10% of cocoa paste (CCN51 or JL2), previously pulverized. The mixture was pelletized and subsequently dried in 40 °C oven for 48 h. The pellet diet was stored at 4 °C until used (maximum 7 days later).

Female Wistar rats (5-week-old at arrival, n=24) were obtained from Janvier Labs (Saint-Berthevin, France) and housed (2 rats per cage) at the Animal Experimentation Unit (UEA) in the Diagonal Campus of the Faculty of Pharmacy and Food Science (University of Barcelona) in polycarbonate cages containing bedding of large fibrous particles (Souralit 1035, Bobadeb S.L., Santo Domingo de la Calzada, Spain) under controlled conditions of temperature and humidity, in a 12:12h light/dark cycle. The animals were randomly distributed into three groups (n = 8 animals/each): the reference group (REF), which was fed with the standard diet; the CCN51 group, which was fed the diet enriched with CCN51 cocoa; and the JL2 group, in which animals were fed the diet enriched with JL2 cocoa. Chow and water were administered *ad libitum* and their intake were monitored three times per week throughout the study.

All animal procedures were conducted in accordance with the institutional guidelines for the Care and Use of Laboratory Animals (EU-Directive 2010/63/UE).

2.2.2. Oral Glucose Tolerance Test

After 26 days of diet, rats were fasted for 6 h with no access to food but access to water. Then, a glucose solution (2 g/kg body weight) was orally administered. Blood samples were obtained from the femoral vein at 0, 15, 30, 60, 90, and 120 min, and blood glucose levels were determined using a glucometer (Adia, Brussels, Belgium).

2.2.3. Sample Collection and Processing

After four weeks of nutritional intervention, the visceral fat from the right half of the body was excised and immediately weighted. Moreover, pH of fecal samples obtained at the end of the study was measured using a surface electrode (Crison Instruments, S.A., Barcelona, Spain). The water content of fecal samples at the end of the study was also measured.

2.3. Statistical analysis

The Student-T test was used for statistical analysis. Significant differences were established at $p < 0.05$.

3. Results and Discussion

3.1. Peruvian Cocoa Composition

The content of total phenolics, total flavonoids, catechin and epicatechin differed between the three cocoa samples (**Table 1**). In particular, JL2 was the population with the highest content of catechin compared to both CCN51 and VRAE99 cocoas ($p < 0.05$). The content of total phenolics was also higher in JL2 compared to CCN51 population ($p < 0.05$);

and the total flavonoid measured as catechin equivalents compared to VRAE99 population ($p < 0.05$). The epicatechin content also differed the CCN51 being the one with the highest content followed by the VRAE99 ($p < 0.05$).

With regards to the antioxidant capacity (**Table 1**), JL2 cocoa had the highest capacity compared to CCN51 and VRAE99 ones ($p < 0.05$), which showed a similar antioxidant capacity.

Table 1. Content of total polyphenols and flavonoids and antioxidant capacity in the cocoa population considered in the study. Results are expressed as mean ± standard error of the mean from the three independent experiments. Values not sharing letters denote significant differences between populations ($p < 0.05$) while values sharing the same letter did not differ.

	CCN51	JL2	VRAE99
Total phenolics (mg GA equivalents/g)	62.31 ± 7.50^{a}	106.62 ± 6.55^{b}	85.47 ± 1.12^{ab}
Total flavonoid (mg catechin equivalents/g)	18.95 ± 0.08^{a}	19.050 ± 0.71^{a}	15.70 ± 0.08^{b}
Catechin (mg/g)	0.41 ± 0.067^{a}	0.63 ± 0.004^{b}	0.44 ± 0.001^{a}
Epicatechin (mg/g)	0.99 ± 0.001^{a}	0.91 ± 0.008^{b}	0.94 ± 0.002^{c}
IC_{50} values of DPPH assay (μM)	127.56 ± 2.58^{a}	106.85 ± 7.19^{b}	129.53 ± 0.75^{a}

3.2. Effect of Peruvian Cocoa Populations on Glucose Tolerance Test

After a 6-hour fasting period, a glucose tolerance test was performed (**Figure 1**). At 15 min after glucose administration, an increase in blood glucose levels was observed in all experimental groups, with no differences among them. Values gradually returned to baseline. At 90 min, animals fed the JL2 diet exhibited higher blood glucose levels compared to the REF group ($p < 0.05$), which normalized after 2 h with no differences between groups.

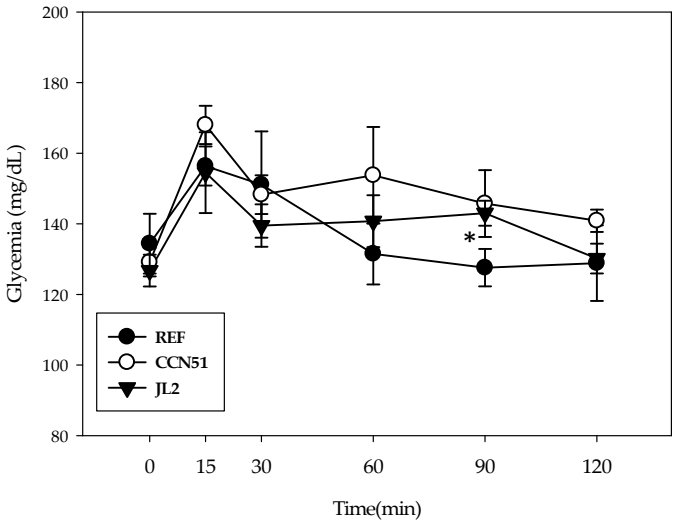

Figure 1. Glucose tolerance test. Results are expressed as mean ± SEM (n=8/group). Statistical differences: *$p < 0.05$ vs REF.

3.3. Effect of Peruvian Cocoa Populations on Body and Organ Weight

Cocoa-enriched diets did not influence the body weight increase (data not shown). However, the intake of both cocoa populations resulted in a lower visceral adiposity at

the end of the study ($p < 0.05$) (**Figure 2**). The reduction in visceral adiposity is in line with that reported in adolescents who followed a flavonoid-enriched diet derived from cocoa-based products [4]. Moreover, flavonoid-enriched diet from conventional cocoa has also evidenced to down-regulate the expression of genes involved in lipid metabolism in colonic samples [5] and to reduce fat deposition [6]. Likewise, anti-obesity activity has also been attributed to cocoa due to their impact on the expression of genes related to lipid metabolism in white adipose tissue [7]. Therefore, further research should be performed in order to identify the compounds responsible for these effects and to elucidate the mechanisms involved.

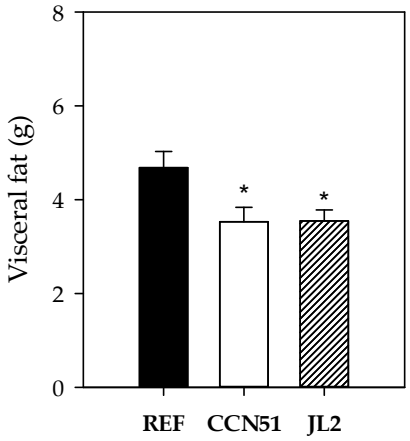

Figure 2. Visceral fat weight (g) at the end of the nutritional intervention for all experimental groups. Results are expressed as mean ± SEM (n=8/group). Statistical differences: *$p < 0.05$ vs REF.

3.4. Fecal variables

Fecal pH and water content were measured (**Figure 3**). We found that the consumption of cocoa-enriched diets (CCN51 and JL2) acidified the fecal samples compared to those obtained from the REF group *($p < 0.05$)* (**Figure 3a**). No differences were observed between the two experimental diets. Similar acidification of fecal and cecal samples was also observed in young rats fed a 10% conventional cocoa-enriched diet for three weeks. In fact, this effect has been linked to the enhancement of beneficial bacteria growth, which was closely related to the concentration of short chain fatty acids [8–10]. Additionally, the shape of microbiota by cocoa polyphenols is also related to the effects on fat index by means of the modulation of the ratio of the two main phyla (*Firmicutes* and *Bacteroidetes*), which has been associated with obesity [11].

Regarding the fecal water content, no differences were observed due to the nutritional intervention; however, CCN51 and JL2 cocoa pastes showed a tendency to reduce their percentage compared to the REF group (**Figure 3b**).

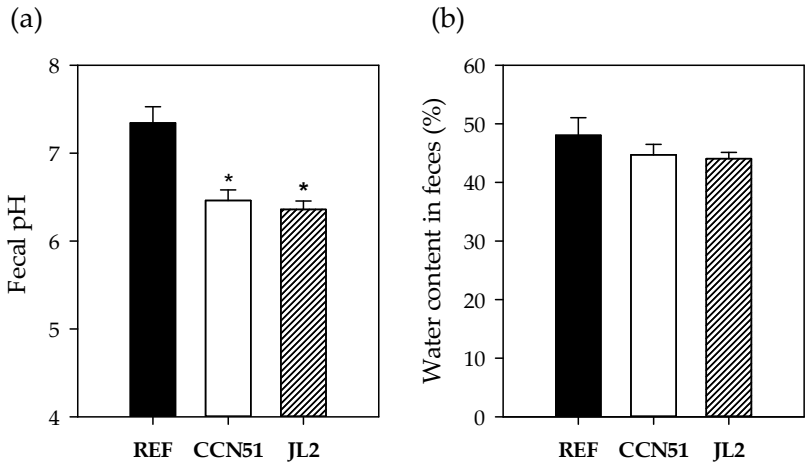

Figure 3. (a) pH and (b) water content in feces at the end of the nutritional intervention for all experimental groups. Results are expressed as mean ± SEM (n=8/group). Statistical differences: *$p < 0.05$ vs REF. .

5. Conclusions

From the "Chuncho" Peruvian cocoa populations used here, JL2 was the cocoa with the highest content of polyphenols and the highest antioxidant activity. However, the intake of diets containing 10% of CCN51 or JL2 cocoa produced same effects on glycemia, visceral fat and fecal variables in young Wistar rats.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethical Committee for Animal Experimentation of the University of Barcelona and the Catalonia Government (CEEA/UB ref. 517/18 P2 and DAAM 10615, respectively), in full compliance with national legislation following the EU Directive 2010/63/EU for the protection of animals used for scientific purposes.

Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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