Effect of avocado honey on anthropometric and biochemical parameters in healthy subjects: a pilot randomised controlled trial

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ABSTRACT

The monofloral avocado honey (AH), characterized by its dark amber color, contains a higher level of bioactive compounds than the multi-floral honey; these characteristics qualify it as a possible sweeter alternative for domestic use. Therefore, the purpose of the present study was to assess the effect of the consumption of avocado honey (25 g/day) on anthropometric and biochemical parameters in healthy subjects for 4-weeks. Thirteen healthy subjects (control group: n = 7 or honey group: n = 6) aged 25–50 years, with BMI (body mass index): <25 kg/m2 completed the study (ClinicalTrials.gov: NCT04572607). There was no significant difference in BMI and body fat percentage. Likewise, no effects on fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were detected. These data suggest that AH might be as effective as a functional food or natural sweeter without negatively influencing anthropometric and biochemical parameters.

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Efecto de la miel de aguacate sobre los parámetros antropométricos y bioquímicos en sujetos sanos: un ensayo piloto controlado y aleatorizado

RESUMEN

La miel de aguacate monofloral (AH), caracterizada por su color ámbar oscuro, contiene un nivel de compuestos bioactivos superior al de la miel multifloral. Estas características la califican como una posible alternativa edulcorante para uso doméstico. Por ello, el presente estudio se propuso evaluar el efecto que conlleva el consumo de miel de aguacate (25 g/día) durante cuatro semanas en los parámetros antropométricos y bioquímicos de sujetos sanos. Trece sujetos sanos (grupo de control: n = 7 y grupo de miel n = 6), de entre 25 y 50 años, cuyo BMI (índice de masa corporal) era <25 kg/m2 completaron el estudio (ClinicalTrials.gov: NCT04572607). Se constató que no se presentaron diferencias significativas en el BMI ni en el porcentaje de grasa corporal. Tampoco se detectaron efectos sobre la glucemia sanguínea en ayunas (FBG), los triglicéridos (TG), el colesterol total (TC), el colesterol de lipoproteínas de alta densidad (HDL-C) o el colesterol de lipoproteínas de baja densidad (LDL-C). Estos datos sugieren que la AH podría ser tan eficaz como alimento funcional o edulcorante natural, sin influir negativamente en los parámetros antropométricos y bioquímicos.

1. Introduction

According to the Codex Alimentarius, honey is the natural sweet substance produced by Apis mellifera bees from the nectar of plants (“Codex Alimentarius information on honey”, 2001). Among natural honey, the monofloral avocado honey (AH), characterized by its dark amber color, contains a higher level of bioactive compounds than the multi-floral honey (Serra Bonvehi et al., 2019). The sweetness of honey is due to the presence of carbohydrates in the form of monosaccharides (fructose (38%) and glucose (31%) are the main contributors) (Gulzar & Tajamul, 2014) and disaccharides. Furthermore, honey contains amino acids, vitamin B, vitamin B6, vitamin C, niacin, folic acid, minerals, iron, zinc, antioxidants (Denisow & Denisow-Pietrzyk, 2016), and phenolic compounds that have been related to positive health effects (Kavanagh et al., 2019). Furthermore, it is characterized by being a sweeter with a medium glycemic index (Ramli et al., 2018), it contains greater sweetness (Meo et al., 2017), and prebiotic oligosaccharides (Bogdanov et al., 2008), and antioxidant enzymes (Rodríguez et al., 2011). Several in vitro and in vivo studies have demonstrated the protective effect on the cardiovascular, nervous, respiratory, and gastrointestinal systems of honey (Al-Waili, 2003; Alvarez-Suarez et al., 2013). For instance, honey has been shown to increase antioxidant status (Toth et al., 2016) and improve blood lipid profiles in healthy, overweight, and hyperlipidemic patients (Al-Waili, 2004; Yaghoobi et al., 2008). These characteristics qualify it as a possible sweeter alternative for domestic use and the food industry since the literature shows that it has greater nutritional properties than refined sugars (Carocho et al., 2017). However, minimal studies have examined the acute and the relatively long-
term (1 month) effects of honey consumption on anthropometric and biochemical parameters. Al-Waili et al, demonstrated an improvement in the blood lipid profiles of healthy subjects and patients with diabetes and dyslipidemia after consuming honey (1.2 g CHO/kg body weight/day) for only 14 days (Al-Waili, 2003). Additionally, Rasad et al, demonstrated that honey (70 g/day) could favorably alter blood lipids relative to sucrose in a cohort of young adults (Rasad et al., 2018). The World Health Organization (WHO) advises restricting added sugar intake to less than 10% of the total daily energy intake (Directriz: ingesta de azúcares para adultos y niños: resumen, 2015), that for a 2000 kcal diet is 50 g/day and an ideal consumption of 5% per day representing 25 g. Therefore, the purpose of the present study was to assess the effect of the consumption of avocado honey (25 g/day) on anthropometric and biochemical parameters in healthy subjects for 30 days and analyze addition to analyzing its viability before carrying out a study with a more significant number of samples.

2. Materials and methods

2.1. Recruitment/screening procedure

This protocol was approved by the ethics committee of the Facultad de Salud Pública y Nutrición (FaSPyN) with the identifier CE 2/2018–19. Through flyers and internet posting, the participants were recruited from the FaSPyN and the local community, Monterrey, Nuevo León, México. Before visiting the laboratory, subjects were screened to verify they did not smoke, did not have a honey allergy, were not pregnant, had not been previously diagnosed with any disease resulting in metabolic disorders, did not chronically use medications known to affect metabolism, and did not participate in greater than 3 hours of structured exercise per week. Upon meeting these criteria, subjects were invited to the laboratory for further screening. On the other hand, female participants that used some contraceptives were excluded. If subjects chose to participate, they were asked to arrive at the laboratory following an overnight fast (12 h). Upon arrival at the laboratory, subjects were asked to read the informed consent before researchers explained the content. The subjects were advised to ask questions for clarification. After subjects signed the informed consent, researchers randomly assigned them to begin the study in one of two groups (control group or honey group). All experimental procedures were conducted according to the guidelines laid down in the Declaration of Helsinki of 1975, revised in 2013, and all procedures involving human subjects were made under the regulation of the General Law on Health Research. Adherence to the protocol was enhanced by frequent contact with the participants by telephone and the provision of gift incentives and was registered at ClinicalTrials.gov with the identifier NCT04572607.

2.2. Study design

Figure 1 shows the flow diagram of the participants from enrolment to analysis. Initially, 18 healthy participants (BMI <25 kg/m²) between 25–50 years of age were recruited, of

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Figure 1. Flow diagram of participant recruitment. Initially, 18 healthy participants (BMI <25 kg/m²) between 25–50 years of age were recruited, of which 13 subjects (male [n=5]) and female [n=8]) completed the study.

Figura 1. Diagrama de flujo del reclutamiento de participantes. Inicialmente, se reclutaron 18 participantes sanos (BMI <25 kg/m2) de entre 25 y 50 años de edad, de los cuales 13 sujetos (hombres [n=5] y mujeres [n=8]) completaron el estudio.
which 13 subjects (male [n = 5]) and female [n = 8]) completed the study. The participants were allocated randomly to either the control group (n = 7) or the honey group (n = 6). Subjects of the honey group consumed 25 g of honey each day for 4 weeks. Participants of both groups did not undergo a special diet regimen, drug therapy, or change in their lifestyle, such as physical exercise, during the study period. However, dietary recommendations were made in both groups to regulate added sugar intake to less than 5% of the total daily energy intake. A 3- day food record, including two weekdays and one weekend day, was used to assess the habitual food intake of the participants before initiation of the trial and repeated each week of intervention. Also, dietary intake was calculated using the database Food Processor® (Version. 10.3.0. ESHA Research, USA), including nutritional information on Mexican foods when they were not on the list. Compliance with the consumption of honey in the intervention group was very high, with 95% of all servings being consumed during the 30-day trial. Compliance was measured by a weekly control call by a research nutritionist and verified the consumption of the samples.

2.3. Test food

Avocado honey was purchased from Hermes Honey S.A. de C.V. (Aguascalientes, México). Mexican company certificated by USDA organic and organic farming EU (https://hermeshoney.com/en/quality/). The honey samples were collected during the harvesting year 2019. Twenty-five grams of honey were dosed in sterile bottles and grouped into 30 samples to facilitate the daily intake by participants, subsequently was storage in the dark until tested (Suárez Vargas et al., 2013). Before delivering honey samples to subjects, they were capable of consuming honey and storage. As follow, the chemical composition of honey is shown in Table 1.

2.4. Anthropometric measurements

All anthropometric variables were measured using a standard methodology (Regecová et al., 2018). Physical measurements, including weight, height, and waist circumference (WC), were measured for all respondents by trained interviewers during the face-to-face interview. Height in meters (m) was measured to the nearest 0.1 centimeters (cm) using a stadiometer (SECA 264, Germany). The Body fat percentage was measured by InBody 120, and BMI was obtained from the weight in kg (SECA scale) divided by height-squared in m.

Table 1. Chemical composition of avocado honey.

| Macronutrient | 100 gr | 25 gr |
|---------------|--------|-------|
| Fructose      | 38     | 9.4   |
| Glucose       | 31     | 7.75  |
| Water         | 17.1   | 4.2   |
| Maltose       | 7.2    | 1.8   |
| Calories      | 308    | 77    |

Source: Supplier Hermes Honey S.A. de C.V.
Fuente: Proveedor Hermes Honey S.A. de C.V.

2.5. Blood samples

Fasting blood samples were collected at the beginning of the study and repeated after 4-weeks. 25-gauge butterfly needle in 13 mL vials containing gel separator and clot activator was used (Vacutest Kima, Arzegrande (PD) Italy), allowed to clot for 15 minutes, and centrifuged at 1200 g for 10 minutes at 4°C The serum was stored at – 80°C for batch analysis.

2.6. Biochemical analysis

Blood samples were collected in the morning after an overnight fast from each subject. Laboratory data, including a complete fasting lipid profile comprising total cholesterol (TC), HDL-C (HDL), LDL-C (LDL), triacylglycerol (TG), and fasting blood glucose (FBG), were quantified by spectrophotometry using biochemistry analyzer A25.

2.7. Statistical analysis

Data were reported as mean ± standard deviation (SD). An independent t-test was used to analyze the difference in pre-intervention markers between the control group and the honey group. The differences between pre-and post-intervention in each group were assessed by paired t-test. A value of p < 0.05 was considered statistically significant. Results were analyzed using IBM SPSS software (Version 25.0).

3. Results

3.1. Subjects

Thirteen healthy subjects (BMI < 25 kg/m²; aged between 25 and 50 years; control group (n = 7) or honey group (n = 6) of the 18 that were recruited completed the study and were included in the statistical analyses. Four participants dropped out before completing the study: three subjects did not meet the inclusion criteria and, two were voluntarily abandoned. Participants followed instructions and adhered to the study protocol, as reflected in the small number of reported missed samples throughout the study. Overall, 95% of the honey samples were reportedly consumed. Both the control group and the honey group did not show significant results in terms of sugar consumption, which represents a moderate consumption according to the WHO, which recommends less than 10% of total energy intake from free sugars. Only the waist-to-height ratio (WHR) was significantly higher in the control (0.5) than honey group (0.47) (p = 0.035, Student’s t-test). The rest of the Baseline characteristics showed no significant differences between the honey and control group (Table 2).

3.2. Dietary intake

Statistical analyses were performed on the dietary intake of 13 subjects (Table 3). Regarding total energy, no significant differences were found between day 0 (baseline) and 4-weeks in both the control and honey groups. The above may be due to all subjects receiving identical training about dietary intake, mainly focused on maintaining the intake of each macronutrient. The consumption of added sugars was
3.3. Anthropometric parameters

Other parameters like anthropometric factors were also analyzed (Table 4). Compared with control group, honey intake did not significantly influence the values of the body weight (baseline: 57.2 ± 11.3; 4 weeks: 56.8 ± 11.2 mg/dL, p = .28). At the beginning of the study, the body weight showed a similar level in both the control and honey groups (control: 57.1 ± 12.7; honey: 57.2 ± 11.3 kg). Since there was no change in body weight in both groups, the BMI indicate similarly data in control (baseline: 21.2 ± 2.2; 4 weeks: 21.1 ± 2.0 Kg/m², p = .58) and honey group (baseline: 22.3 ± 2.3; 4 weeks: 22.2 ± 2.28 Kg/m², p = .26). Regarding body fat percent, the control group maintained levels after 4-weeks without significant difference (baseline: 21.9 ± 7.2; 4-weeks: 21.7 ± 6.8%, p = .43). Although the honey group shown 0.9% less of body fat compared to beginning value, it was not significant (baseline: 28.2 ± 7.0; 4-weeks: 27.3 ± 7.4%, p = .25). There was no significant difference in waist and hip circumference compared with baseline data in the control and honey groups.

4. Discussion

As we have mentioned above, honey is a naturally sweet product that, compared to sugar, has a complex composition. When we talk about honey composition, many aspects should be considered because botanical and geographical origins determine the specific composition and properties of all types of honey (Bobiš et al., 2018). Additionally, honey could present a lower glycemic index and energetic values than sugar.

In the current study, the honey dose of 25 g per day was analyzed, considering the WHO’s recommendation about the consumption of sugars. We observed that honey dose did not negatively affect the level of FBG and the lipid profile (TG, LDL-C, TC, and HDL-C) of healthy participants; additionally, some anthropometric parameters, such as weight, body fat percent and, BMI, neither were affected. Although

3.3. Biochemical parameters

Biochemical parameters are shown in Figure 2. The baseline and final values of FBG were not different in control (baseline: 86.0 ± 4.1; 4 weeks: 88.8 ± 4.6 mg/dL, p = .06) and honey group (baseline: 86.9 ± 4.2; 4 weeks: 88.1 ± 4.6 mg/dL, p = .08). After intake honey during 4-weeks no significant change was found in TC (baseline: 157.6 ± 24.6; 4 weeks: 160.1 ± 30.4 mg/dL, p = .50), HDL-C (baseline: 52.3 ± 9.7; 4 weeks: 51.8 ± 10.4 mg/dL, p = .84), LDL-C (baseline: 90.1 ± 18.2; 4 weeks: 91.7 ± 24.2 mg/dL, p = .71) and TG (baseline: 72.5 ± 23.4; 4 weeks: 82.8 ± 28.3 mg/dL, p = .28).

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previous studies, either model animals or clinical trials, have demonstrated the effect of honey consumption on lipid profile and FBG, the association between honey consumption and its effect on healthy people as a functional food has not been extensively investigated by clinical trials. Our results are similar to those reported by Münstedt et al. (2009) because the authors did not observe significant differences in cholesterol or triglycerides when participants...
consumed honey (Münstedt et al., 2009); however, the lack of standardization of measurements and variety of methods used as well as differences in the study population and sample size might cause these inconclusive findings. In another study developed by Bekkayev (2016), 10 g of natural honey per day for 70 days were analyzed, finding that lipid profile was improved in patients with type 2 diabetes. However, the changes were not statistically significant, which was probably because of the lower dose of honey. Therefore, lower doses of honey could not negatively affect the lipid profile and fasting blood glucose, as in this current study (Patti et al., 2018).

On the other hand, a clinical trial evaluated the consumption of 40 g of honey per day for 4-weeks, finding that honey consumption improved the levels of total cholesterol, LDL-c, triglycerides, and HDL-c in subjects with obesity of different ethnic groups and both genders significantly (Mushtaq et al., 2011). Besides, it has been reported that consuming a diet containing 25% energy as honey slightly significantly decreases postprandial blood glucose. However, it does not significantly increase postprandial plasma triglycerides compared to an isocaloric starch diet (Campos et al., 2017). Recently, it has been informed that 70 g of natural honey per day for 6 weeks significantly decreases total cholesterol, triglycerides, and LDL-c, but it significantly increases HDL-c in healthy young subjects compared to sucrose consumption (Rasad et al., 2018). Indeed, it has been reported that 1.2 g/kg of honey per day for 4-weeks does not significantly affect total cholesterol, LDL-c, and HDL-c in healthy subjects of both genders (Al-Tamimi et al., 2020). In this sense, most of the effects shown in previous studies on honey consumption could be associated with the variability of the doses used, since they range between 10 and 75 grams per day, rather than with the composition of the brand of honey.

Since honey composition is complex due to antioxidants, such as beta-carotene, vitamin C, uric acid, and many minerals, all of them could play a role in lipid and glucose metabolism (Bahrami et al., 2008). In this sense, the antioxidants might be responsible for their biological activity. Nevertheless, the honey content of fructose and glucose can have an important role in its effect (Miguel et al., 2017; Rasad et al., 2018). Also, insulin secretion can be increased by honey consumption, which could increase the absorption of glucose and lipid synthesis, reducing lipolysis, which can lower lipid and glucose levels in the serum (Rasad et al., 2018).

The regulatory mechanisms of honey on obesity are still being studied due to the large amount of constituents, such as oligosaccharides (1.5–2.0%), free organic acids (0.2–2.0%), phenolic acids (1.5–4.2%) and flavonoids (1.2–2.5%) (R & EM, 2016); some beneficial properties could be attributed to polyphenols which are known to reduce body weight and fat mass. An animal study reported caffeic and chlorogenic acid supplementation (0.02% w/w) for 8 weeks in mice fed a high-fat diet significantly reduced body weight by 8% and 16%, respectively. In this experiment, supplementation with caffeic and chlorogenic acid also reduced plasma leptin, indicating the alleviation of leptin resistance (Cho et al., 2010). Besides, the gallic acid supplementation (250 μM 48 and 72 h) showed an increase in Fas (CD95)/Fas Ligand (Fasl; CD95 L) and p53 expression promoting the process of lipoysis in 3T3-L1 preadipocytes (Hsu et al., 2007). Also, various studies reported that the administration of fructo-oligosaccharides promotes a reduction in weight gain and energy intake in animal models (Erejuwa et al., 2011); these fructans also decreases malic enzyme activity, a lipogenic key enzyme in providing NADPH for fatty acid elongation by FAS (Daubioul et al., 2000).

The effectiveness of clinical study results is based on the training of the participants before the start of the trial. The honey group reported 98% compliance in the intake of the samples. It was recommended to avoid consuming sugary drinks and sweeteners added to food the above, not to affect the final results. The nutritional recommendations provided to the study group contribute to education on proper nutrition. In this sense, trends in future sweetener use will likely be influenced by increasing obesity prevalence and consumer demand (Edwards et al., 2016).

Therefore, dietary intake and questions on dietary assessment for nutritional epidemiology play an important role in the study of food customs (Assmann et al., 2015). In our study, we carried out a food frequency and a 3-day food record, including two weekdays and one weekend, to assess the habitual food intake of the participants before to initiation of the trial and repeated each week of the intervention. The information obtained from the food record made it possible to control energy intake, as well the consumption of added sugars that remained under 5% according to the WHO guidelines recommend limiting free sugars intake to less than 10% of total energy intake to prevent excess body weight, and less than 5% for additional health benefits (Cediel et al., 2017). Another advantage of the dietary record was identifying that the intake of proteins, carbohydrates, and fat did not change with the consumption of 25 g/day of honey. The main concern about sugars is the disproporionated contribution to dietary energy intake, as non-nutritive sweeteners (e.g. aspartame, stevia) are increasing in popularity. However, the assumed role in facilitating body weight management is far from established (Liauchonak et al., 2019). Honey has been safely consumed for generations, and although it contributes to energy intake, this sweetener tends to have lower glycemic potency than refined sugars (Deibert et al., 2009). Moreover, it contains a plethora of nutrients and bioactive compounds (e.g. polyphenolics) that may be of potential benefit to health (Alvarez-Suarez et al., 2010). In this trial, the absence of a significant decrease in body weight indicates an energy balance in both groups despite the consumption of avocado honey. Even so, showing that free sugars, when consumed in approximately 5% of the total energy intake, do not generate an increase in body weight in healthy subjects. The above has also been reported by Prinz et al (Prinz, 2019) supposing that changes in body weight occur when intake of sweeteners along with the rest of the diet exceeds total energy intake. Likewise, Rashid et al. showed that the body weight did not change after intake of honey (30 g/day) for 30 days. Weight gain is not only generated by the intake of caloric sweeteners but also originates when the caloric sweetener, together with the sum of all the foods ingested, exceeds the total caloric expenditure during a time (Rashid et al., 2019).

However, our study presents some limitations which should be considered. First, residual confounding cannot be excluded, despite several adjustments for a wide range of confounding factors. Second, we were unable to examine the effects of honey consumption on a wide number of
subjects because 18 subjects were recruited, and 13 subjects completed the clinical trial. Third, due to the world health problem, the coronavirus pandemic, we did not assess the effect of honey consumption on other biomarkers of inflammation, antioxidant capacity, oxidative stress, and inflammatory cytokines, such as C-reactive protein, adiponectin, which may also be regarded as an important endocrine regulator of the obesity (Castro et al., 2016). However, the strength of this study is generalizability to the community, it is done in healthy individuals, in addition, we considered the WHO’s guidelines for sugar consumption. According to the percentage of compliance in taking the samples and the results obtained in the parameters analyzed, we consider the design study feasible to increase the sample size, in addition to analyzing various biomarkers of inflammation mentioned above.

In conclusion, our results suggest that 25 g of honey per day for 4-weeks does not negatively affect blood glucose levels, lipid profile, weight, body fat, and BMI. This dose of honey does not influence the intake dietary. Therefore, these results indicated that natural avocado honey might be effective either as functional food or as a natural sweeter. However, to confirm our findings, further studies are needed, particularly one with larger sample sizes, and examine biomarkers of inflammation and oxidative stress to establish a mechanism of action.

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Author contributions
A.M.F.B; H.C.G; E.R.L; J.M.L.D; B.A.R.R and M.H.S conceived and designed the experiment, A.M.F.B performed the experiment, A.M.F.B; H.C.G and M.H.S analyzed the data and wrote the paper. All authors read and improved the manuscript.

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No potential conflict of interest was reported by the author(s).

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