Apple cider vinegar modulates gut microbiota, improves lipid profile and attenuates tissue damage in rats with diet induced obesity.

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Obesity is well-recognized as a global epidemic and is associated with various co-morbidities. The intestinal microbiota seems to play a major role in the development of this pathology. In addition, environmental factors can modulate the composition of the intestinal microbiota and promote or prevent the development of metabolic abnormalities. This study is conducted to verify the impact of apple cider vinegar on lipid profile, intestinal structure and modulation of gut microbiota in Wistar rats subjected to a cafeteria diet. 24 male adult Wistar rats are divided into 3 equal groups. A witness group submitted to standard laboratory diet and two groups subjected to cafeteria diet; one receives a daily gavage of ACV (7 mL/kg/day) for 90 days. Anthropometric measurements are performed on d0 and d90. At the end of the experiment, the animals are sacrificed, the blood is collected for biochemical assays, the jejunal fragments are removed for histological study and the contents of the colon and feces are freshly collected under sterile conditions for microbiological study. Our results show that after 90 days of experimentation, the ACV supplementation leads to a highly significant (p≤0.001) decrease in weight gain and BMI compared to the RC group, correlated with an improvement of lipids profile and a highly significant decrease (p≤0.001) of Firmicutes (Enterobacteriaceae and Clostridium) both in colon and faeces. In conclusion, ACV might have a positive impact on the control of weight gain by modulation of gut microbiota, thus prevent positive energy balance and reverse over- weight and obesity.

Key words: apple cider vinegar; obesity; hypercholesterolemia; anthropometry; intestinal microbiota

INTRODUCTION
The epidemic of obesity around the world has become an important public health issue, with serious psychological and social consequences (Hill, 2012; Apovian, 2016). Obesity is a pathological risk factor with various co-morbidities, including hypertension, insulin resistance and other components of the metabolic syndrome (Haslam and James, 2005).

Obesity is associated with an imbalance in energy intake and expenditure that is characterized by excessive body fat accumulation. Obesity is also a multifactorial condition which is influenced by a complex interplay of genetic, epigenetic and is strongly associated with the behavior of the persons in their environment (Drong et al. 2012)

It is measured through the Body Mass Index (BMI), a simple index of weight–height relationship that indicates amount of body fat used to classify overweight and obesity in adults (Burslem, 2004)
The gut microbiota is known to play an important role in energy homeostasis and the control of body weight (Bakker, 2015; Gérard, 2016). In the human gut, predominant microbiota belong to the phylum, Firmicutes and Bacteroidetes, which account for more than 90% of the overall phylogenetic types (Eckburg et al. 2005).

It is now well established that diet has a great impact on the composition of the gut microbiota and the patterns of metabolic functions of the microorganisms (Flint and Lobley, 2007; Yatsunenko et al. 2012). The Western-style diet characterized by high-fat and low-fibre content is considered to be one of the main factors that contribute to gut microbiota dysbiosis.

Several studies have documented that diet-induced obese mice show an increase in the proportion of gut microbiota members of Firmicutes and a decrease in Bacteroidetes when compared with their lean relatives (Turnbaugh et al. 2008). Studies in humans have also associated fewer Bacteroidetes and more Firmicutes in obese people than in lean control subjects (Ley et al. 2006).

In addition to an increased ratio of Firmicutes vs. Bacteroidetes, obesity has been associated with reduced bacterial diversity and enrichment of genes related to lipid and carbohydrate metabolism (Turnbaugh, 2009).

The aim of the pharma-biotic industry is to manipulate the intestinal microbiota for therapeutic purposes. Nowadays, several possibilities are envisaged in order to shape the microbiota by modifying the diet, probiotics (Cani, 2006), faecal transplantation (Lagier and Raoult, 2016) but also phenolic compounds found in our daily diet (Del Rio et al., 2013).

There are promising reports of different anti-obesogenic phytochemicals (Bahmani et al., 2016; Rios-Hoyo and Gutiérrez-Salmeán, 2016; Farhat et al., 2017). Vinegar has long been consumed as a cooking ingredient and used as a folk medicine (Kondo et al., 2009a). Currently, various types of vinegar originating from different crops or fruits are consumed throughout the world (Kondo et al., 2009b). Vinegar has been reported to have antibacterial (Sengun and Karapinar, 2004), cardiovascular protective (Honsho et al., 2005) and antitumor effects (Mimura et al., 2004). Previous findings showed that apple cider vinegar and acetic acid (the main ingredient of vinegar) affect lipid profile and weight loss (Kondo et al., 2009; Petsiou et al., 2014; Seo et al., 2015), and have a strong antioxidant activity due to the phenolic compounds that compose it (Dávalos et al., 2005).

The present study investigates the preventive effects of apple cider vinegar on modulating the composition of the gut microbiota in order to positively influence the health, which can appear today as a new therapeutic way.

MATERIALS AND METHODS

Materials

The apple cider vinegar (ACV) used in our study is a vinegar marketed in Algeria.

Methods

Animals

Twenty-four old rats male Wistar with an initial weight of 140 ± 05g were kept in a laboratory environment of light-and-dark cycles and divided randomly into 4 groups (8 rats in each group): Standard Diet (SD) subject to the standard laboratory diet (normolipidic), Cafeteria Diet (CD) subject to the cafeteria diet and Cafeteria Diet supplemented with ACV (CDV) at the dose of 7ml/kg via orogastric feeding tube. All animals had free access to water.

At the end of the experiment, the animals are sacrificed. The blood is collected by cardiac puncture, recovered in heparinized tubes, centrifuged at 3500 rpm for 15 minutes at 4 °C. Serum is recovered and stored at -20°C for biochemical assays. The jejunal fragments are removed for histological study and the contents of the colon and faeces are freshly collected under sterile conditions to verify their microbial quality by a microbiological study.

Induction of obesity

In order to generate a significant weight gain in rats and provide a reliable model of obesity, we subjected the animals to a "cafeteria" diet. This diet has the advantage of being similar to that of the majority of human cases in which obesity is prompted by deliberate overconsumption of foods rich in fat and calories. It facilitates the interpretation of observed metabolic changes, because they are not masked by other abnormalities associated with genetic obesity.

The cafeteria diet offers animals a variety of palatable, high-fat, simple-carbohydrate foods with the following composition: 50% standard diet and 50% sausage-cookies-cheese-chips-chocolate - peanuts in the proportions 2:2:2:1:1:1 (Darimont et al., 2004).
**Anthropometric determination**

In order to study the effects of obesity on anthropometric parameters, the following parameters were determined for all rats at the beginning (day 0) and at the end of the experiment (day 90): weight, and body length (nose-anus length). The Body Mass Index (BMI) is calculated according to the following formula: 

$$\text{BMI} = \frac{\text{body weight (g)}}{\text{length}^2 (\text{cm}^2)}$$

**Plasma lipids profile**

Plasma lipids determination [total cholesterol (TC), triglycerides (TG), and high-density apolipoprotein-cholesterol (HDL-c)] is carried out by enzymatic colorimetric method (Kit Spinreact, Girona, SPAIN). The low-density lipoprotein-cholesterol (LDL-c) were calculated by the Friedewald formula (Friedewald et al., 1972). The atherogenic risk assessment criteria are based on the lipid balance and the atherogenic index (AI) was calculated according to the formula [AI = TC / HDL-c].

**Microbiological analysis**

To favor better growth of the bacteria, one milliliter of each sample (colon and faeces) was mixed with nine milliliters of sterile physiological saline solution. The homogenate was serially diluted and the appropriate dilutions were surface plated on specific media : MRS agar (pH= 5.4 ; pH = 6.8) (De Man et al., 1960), LM agar (liver meat) and MC agar (MacConkey). Plates were then incubated at 37 °C for 48 h. A growth between 30 and 300 colony-forming units per gram was taken as positive culturing. Colonies presenting different morphologies were randomly selected from each plate and were purified by repeated streaking on the appropriate agar media. Selected isolates were evaluated by catalase test, Gram staining, and cell morphology.

**Histological analysis**

For histological analysis, the intestinal fragments (jejunum) were fixed in a buffer solution containing 10% formalin, after embedded in paraffin. Next, 4 µm sections were prepared and stained with hematoxylin and eosin. The stained areas were observed using an optical microscope (Olympus, France) at a (100 ×) and (400 ×) magnification. Slides were examined by a pathologist who was not aware of the grouping.

**Statistical analysis**

All data are expressed as mean ± standard error (SE). The comparison of the means between the three groups of rats (under a standard diet, cafeteria diet or cafeteria diet supplemented with apple cider vinegar) was performed using ANOVA test. The differences are considered significant at p <0.05.

**RESULTS**

**Effect of ACV on changes in body weight and anthropometric parameters**

As shown in Figure 1, the average weight of the different groups does not show any significant difference at the beginning of the experiment. However, the cafeteria diet resulted in a very significant increase (p <0.001) of the body weight of the rats from the 21th day, which became very important after 28th day of diet. At 14th day, ACV caused a high significant decrease in body weight (p <0.001) until the end of the experimentation.

**Effect of ACV on anthropometric parameters**

The anthropometric parameters reveal a highly significant increase (p <0.001) in weight gain in the CD group compared to the SD group correlated with a highly significant increase (p <0.001) in BMI. However, a highly significant decrease (p <0.001) is noted as well for weight gain and BMI in the CDV group compared to the CD group (Table 1).

**The effect of ACV on serum lipids profile**

The effect of ACV on serum lipids profile is shown in Table 2. Rats subjected to cafeteria diet and treated with ACV are compared to untreated rats. ACV leads to a very significant decrease (p <0.01) in TC (29.60%), and in a highly significant decrease (p <0.001) in TG (49.89%), LDL-c (58, 08%) and VLDL (49.89%), in addition a highly significant increase (p <0.001) in HDL-c (33.93%) is noted with protection against atherogenic risk (60.76%).
Bouderbala et al., Effect of Apple cider vinegar in obese Wistar rats

Figure 1. Body weight changes during 90 days of experimentation relative to different groups

Each value represents the mean ± SE (Standard error), n = 8

Significant threshold value difference between SD, CD and CDV: * P <0.05; ** P <0.01; *** P <0.001

*: Significant difference between lots: SD vs CD; SD vs CDV

#: Significant difference between the two experimental groups CD vs CDV

Table 1: General anthropometric characteristics of rats of different groups at the beginning and the end of the experiment

<table>
<thead>
<tr>
<th>Diets</th>
<th>SD</th>
<th>CD</th>
<th>CDV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d0</td>
<td>149.63 ± 1.57</td>
<td>142.25 ± 1.14</td>
<td>147.12 ± 1.42</td>
</tr>
<tr>
<td>d90</td>
<td>283.62 ± 1.52</td>
<td>389.75 ± 4.53***</td>
<td>257.50 ± 0.86###</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Weight gain (g)</strong></th>
<th></th>
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<tbody>
<tr>
<td>d0</td>
<td>134.00 ± 2.26</td>
<td>247.5 ± 1.68***</td>
<td>110.375 ± 1.96***###</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Length (cm)</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>d0</td>
<td>15.41 ± 0.13</td>
<td>15.18 ± 0.14</td>
<td>14.62 ± 0.23</td>
</tr>
<tr>
<td>d90</td>
<td>21.82 ± 0.13</td>
<td>21.82 ± 0.26</td>
<td>21.53 ± 0.12</td>
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</table>

<table>
<thead>
<tr>
<th><strong>BMI (g/cm²)</strong></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>d0</td>
<td>0.63 ± 0.01</td>
<td>0.62 ± 0.01</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>d90</td>
<td>0.60 ± 0.01</td>
<td>0.82 ± 0.02**</td>
<td>0.56 ± 0.01###</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE (Standard error), n = 8

Significant threshold value difference between SD, CD and CDV: * P <0.05; ** P <0.01; *** P <0.001

*: Significant difference between lots: SD vs CD; SD vs CDV

#: Significant difference between the two experimental groups CD vs CDV
Table 2: Effect of ACV on plasma lipids profile.

<table>
<thead>
<tr>
<th></th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td><strong>Plasma Lipids profile</strong></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>54.89 ± 4.08</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>49.35 ± 5.00</td>
</tr>
<tr>
<td>HDL-c (mg/dL)</td>
<td>63.74 ± 2.40</td>
</tr>
<tr>
<td>LDL-c (mg/dL)</td>
<td>63.56 ± 1.37</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>9.87 ± 1.00</td>
</tr>
<tr>
<td>AI</td>
<td>0.82 ± 0.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE (Standard error), n = 8

SD: Standard Diet, CD: Cafeteria Diet, CDV: Cafeteria Diet supplemented with ACV

Significant threshold value difference between SD, CD and CDV: * P <0.05; ** P <0.01; *** P <0.001

*: Significant difference between lots: SD vs CD; SD vs CDV

#: Significant difference between the two experimental groups CD vs CDV

Figure 2: Observation under optical microscope of histological samples of rats’ jejunum colored to hematoxilin-eosin: group SD (a,d), group CD (b,e), groupe CDV (c,f).

Effect of ACV on morphological features of jejunum

Histological sections in the jejunum show an intestinal mucosa with normal villus, whose average height is 45.86 ± 0.71 µm. This mucosa is based on a serosa and a muscularis of normal appearance (Fig 2: a, d). For the CD group, histological and cytological changes are detected: a villus atrophy (25.23 ± 0.71 µm vs 45.86 ± 0.71 µm; p<0.001) with a decrease in the thickness of the muscularis and the presence of lipid vacuoles (Fig 2 : b), localized necrosis of villus, hyperplasia of goblet cells, an important inflammatory infiltrate (Fig 2 : e).

For the CDV group, less important changes than those seen in the CD group are observed: hyperplasia and atrophy of villus (28.23 ± 0.71 µm vs 25.23 ± 0.71 p<0.01), a less important inflammatory infiltrate than observed in the CD group, an hypertrophy of smooth muscle fibers, with decrease in the thickness of the muscularis (Fig 2 : c) and an hypertrophy of goblet cells (Fig 2 : f). The findings of villus length can be seen in...
Figure 3: Villus length (μm) of the different experimental groups.

Each value represents the mean ± SE (Standard error), n = 8
SD: Standard Diet, CD: Cafeteria Diet, CDV: Cafeteria Diet supplemented with ACV
Significant threshold value difference between SD, CD and CDV: * P <0.05; ** P <0.01; *** P <0.001
*: Significant difference between lots: SD vs CD; SD vs CDV
#: Significant difference between the two experimental groups CD vs CDV

Figure 4: Growth of (A) Lactobacillus on MRS agar (pH 5.4), (B) Lactic acid bacteria on MRS agar (pH 6.8), (C) Enterobacteria on MC agar, and (D) Clostridium on LM agar in colon and faeces of different experimental groups.

Each value represents the mean ± SE (Standard error), n = 8
SD: Standard Diet, CD: Cafeteria Diet, CDV: Cafeteria Diet supplemented with ACV
Significant threshold value difference between SD, CD and CDV: * P <0.05; ** P <0.01; *** P <0.001
*: Significant difference between lots: SD vs CD; SD vs CDV
#: Significant difference between the two experimental groups CD vs CDV
Table 3: Morphological and physiological profile of the isolates

<table>
<thead>
<tr>
<th>Shape</th>
<th>Gram</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus</td>
<td>bacillus</td>
<td>+</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>bacillus</td>
<td>-</td>
</tr>
<tr>
<td>Clostridium</td>
<td>coccus</td>
<td>+</td>
</tr>
</tbody>
</table>

**Growth of bacteria**

The overgrowth of *Lactobacillus* shows a very significant decreased (p<0.01) in colon of CD group, and significant decreased (p<0.05) in faeces compared to SD group. While no significant difference is noted between the three groups in faeces.

The results regarding the enumeration of Lactic acid bacteria show a highly significant increase (p <0.001) in both CD and CDV colon's. While no significant difference is noted between the three groups in faeces.

The overgrowth of *Enterobacteria* show a very significant increase (p <0.01) in colon and significant increase (p <0.05) in faeces in CDV group compared to SD group. In contrast, a highly significant decrease (p <0.001) in colon and faeces is noted in CVD group compared to CD group.

The growth of *Clostridium* in colon and faeces show a highly significant increase (p <0.001) in CD group compared to SD group, and very significant increase (p <0.01) in CDV compared to SD group. However, a highly significant decreased (p <0.001) is observed in both colon and faeces in CDV compared to CD group. These results were illustrated in figure 4. The results of the morphological and physiological profile of the isolates are presented in Table 3.

**Discussion**

Obesity, which is primarily attributable to a prolonged imbalance between energy intake and energy expenditure, is a prevalent health issue worldwide and is associated with modernization. Although genetics and environmental factors are major causes of obesity, modern eating habits, especially consuming HFDs, have been identified as the most important reason for the increased risk of obesity (Clarke et al., 2012).

Recently, several natural phytochemicals derived from fruits and vegetables have been demonstrated to suppress obesity and obesity-related metabolic syndrome (Keophiphath et al., 2009; Ueda and Ashida, 2012).

Fruit vinegar (FV) is a kind of beverage and is becoming more popular throughout the world for its potential as a functional beverage (Liu et al., 2012).

In this context, the objective of this study is to evaluate the effect of apple cider vinegar on anthropometric parameters, plasma lipids profile, intestinal microbiota and intestinal histology in adult Wistar rats subjected to the cafeteria diet.

As observed in this current study, CD feeding for 90 days significantly increased the rat body weight, BMI and leads to hyperlipidemia. Our results demonstrated that ACV had potent anti-obesity effects on CD-induced obese rats.

Interestingly, the supplementation with ACV prevents the gain of weight and consequently an important decrease in BMI. We conclude that ACV provide a satiatogenic effect, which is in agreement with Kondo et al., (2009) who demonstrate that acetic acid was considered to be the active ingredient in vinegar that affected body fat reduction and body weight gain.

Improving lipid metabolism is one of the most common strategies for treatment of obesity (Kim et al., 2012). In recent years, natural alternative anti-obesity agents in the form of beverages or tea have been used to treat obesity (Park et al., 2009).

This study revealed that the administration of CD leads to an important increase in serum TC, TG, VLDL, and LDL-c concentrations and significant decrease in HDL-c concentrations after 90 days of experiment. However, ACV improves serum lipids profile by decreasing TC, TG, VLDL, and LDL-c, and increasing HDL-c concentrations. These findings are in accordance with the result of Bárdos et al., (2012) who reported that oral administration of ACV to normal mice induced a significant reduction in plasma TG levels. Furthermore, Fushimi et al., (2006) have reported that acetic acid lowered serum TG in rats fed cholesterol-rich diet. The hypotriglyceridemic effect of AVC might be due to the reduction of hepatic TG storage (Bárdos and Bender, 2012).
In recent years, the effect of modulation of intestinal microbiota by polyphenols has been widely studied. Overall, polyphenols are known to increase the number of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and to reduce the number of harmful bacteria such as *Enterobacteriaceae* and *Clostridium* (Duda-Chodak et al., 2015; Dueñas et al., 2015).

In the human gut, predominant microbiota belongs to the phylum, Firmicutes and Bacteroides. Research on human microbiota points to the role that a diet can play in the development of obesity. Indeed, the composition of microbiota is modulated in response to the diet, in this case a break is observed in favor of Firmicutes (Le Chatelier et al., 2013). On the other hand, the results of the present study show that there is a highly significant increase in Enterobacteria and Clostridium in CD group compared to SD group. In contrast, the CDV group presents a significant lower proliferation rates. Hence, the results obtained are fully in line with Le Chatelier’s conclusion. Also, the results show the involvement of the polyphenols of the ACV in the modulation of the composition of the intestinal microbiota.

Researchers estimate that 90-95% of dietary polyphenols are not absorbed in the small intestine and reach the colon (Del Rio et al., 2013), which could explain the protective effect of ACV against the proliferation of pathogenic bacteria, such as Clostridium in the colon.

CONCLUSION
The results of this study demonstrated that ACV can be used as a functional beverage that regulates body weight, afforded protection against dyslipidemia, atherogenic risk related to obesity and modulate the intestinal microbiota by reducing harmful bacteria like *Enterobacteriaceae* and *Clostridium*, which can be an interesting therapeutic approach.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
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AUTHOR CONTRIBUTIONS
HB performed the animal experiments, tissue collection, data analysis and also wrote the manuscript. CZ performed the histological study. WD and FR contributed to the microbiological study. OK, DS and HK contributed to the experimental design and reviewed the manuscript. All authors read and approved the final version.

REFERENCES


Liu F, He Y, Wang L. 2008. Determination of...
Bouderbala et al., 2009. Effect of Apple cider vinegar in obese Wistar rats


