Evidence suggests that overweight and obesity prevalence have been arising at alarming rates, both in developing and developed countries [1]. In fact, obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems [2]. Several animal models have been used for experimental studies such as Wistar rat. Inducing obesity in rats by a high-fat-diet increases lipid peroxidation markers [3], food intake, body weight, glycemia and adipose tissue [4]. Numerous studies have shown an inverse relationship between the consumption of fruits, vegetables and legumes and cardiovascular disease (CVD) risk factors, including obesity, hypertension, diabetes mellitus and metabolic syndrome [5]. Adoption of a Mediterranean Diet (MD) pattern is associated with reduced mortality and improvements in CVD factor levels that could be explained by the presence of many of the constituents of these foods [6]. Cereals and legumes association can be found in the culinary traditions of many countries: rice and soybeans in the Far East, couscous and chickpea in North Africa, maize and beans in America, wheat, barley and beans in Europe [7].
White lupin (*Lupinus albus*) has been little studied compared to other legumes such as soybeans and chickpeas. White lupin is a protein-rich legume seed and contains negligible amounts of isoflavones. The sweet varieties of lupine have been cultivated for centuries for domestic animal feed, but also for human nutrition [8]. Wheat is a major dietary source of essential minerals and vitamins and of other beneficial bioactive components (phytochemicals and dietary fibers components) [9]. As well, oat, a whole-grain cereal, has been little investigated compared to other cereals. It’s incorporation into a weight loss program diet can improve the lipid profile [10]. Cereals and legumes decrease low density lipoprotein cholesterol (LDL-C) and blood pressure, improve glucose metabolism and endothelial function [11]. In the human diet, legumes and whole-grains are two complementary sources of protein. For these reasons legumes are considered an ideal complement to cereals in vegetarian diets and they gain increasing attention as functional food items. Within the context of the adoption of a healthier diet, it is recommended that legumes consumption should increase in the Western diet [12].

Lecithin-cholesterol acyltransferase (LCAT; EC 2.3.1.43) is a key enzyme for the production of cholesteryl esters (CE) in plasma and promotes the formation of high density lipoproteins (HDL) [13]. LCAT stimulates the supposedly antiatherosclerotic reverse cholesterol transport (RCT) pathway and it has also been recognized to protect LDL from oxidative modification [14]. Importantly however, a proatherosclerotic effect of LCAT on very low density lipoprotein (VLDL) lipids was suggested [15]. HDL plays an important protective role against atherosclerosis. The anti-atherogenic properties of HDL include the promotion of cellular cholesterol efflux and RCT, as well as antioxidant and anti-inflammatory effects [16].

Literature studies have reported the individual effects of cereals and legumes [10, 11] but few is known on their possible combinations in the reduction of many diseases like the risk of coronary heart disease (CHD), type 2 diabetes, obesity. In this context, the purpose of this study was to determine the beneficial effects of legumes and cereals association on hyperglycemia, dyslipidemia, serum HDL\(_2\) and HDL\(_3\) amounts and compositions and LCAT activity in rats fed a high-fat diet.

**MATERIALS AND METHODS**

**Animals and diets**

Male Wistar rats (Iffa Credo, L’Arbresle, Lyon, France), weighing 110 ± 20 g were housed under standard environmental conditions (23 ± 1°C, 55 ± 5% humidity and a 12 h light/dark cycle). Obesity was induced by feeding a high-fat diet (20% mutton fat) during 3 months. Sixteen obese rats were divided into two groups (n = 8) and fed diets containing either 1/3 of white lupin + 2/3 of wheat (wheat-lupin group) or 1/3 of white lupin + 2/3 oat (oat-lupin group) during 28 days. The diets were mixed in our laboratory and their detailed composition is shown in Table 1. Water and food were given *ad libitum* throughout the experiment. Body weight (BW) was followed once a week and food intake was estimated every day. We followed the General Guidelines on the Use of Living Animals in Scientific Investigations (Council of European Communities, 1987) [17].

**Blood and liver collection**

At day 0 (beginning of the experiment), blood was collected from the tail vein of fasting rats in order to verify the high-fat diet effects on glycemia and lipid profile. After 4 weeks of experiment (day 28) and 12 h fasting, between 08.00 and 09.00 h, eight rats from each group were weighed and anesthetized with sodium pentobarbital (60 mg/kg-BW) and euthanized with overdose. Blood was collected from the abdominal aorta into tubes and was centrifuged (1000g for 20 min at 4°C). Liver was rapidly removed, washed with cold saline, blotted on filter paper, and weighed. A serum aliquot was preserved in tubes containing 0.1% Na\(_2\)EDTA and 0.02% sodium azide (to inhibit bacteria growth) for lipids and lipoproteins assays and another aliquot (fresh serum) was used to measure LCAT activity. Liver samples were stored at −70°C until use.

**Table 1. Composition of experimental diets (g/kg diet)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Oat-Lupin</th>
<th>Wheat-Lupin</th>
</tr>
</thead>
<tbody>
<tr>
<td>White lupin(^a)</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>Oats(^b)</td>
<td>450</td>
<td>-</td>
</tr>
<tr>
<td>Wheat(^c)</td>
<td>-</td>
<td>450</td>
</tr>
<tr>
<td>Animal fat(^d)</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn strach(^e)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose(^f)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix(^g)</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mix(^h)</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^a\)The diets were high fat (20% mutton fat), contain 2MJ and were given in powdered form. \(^b\)Exhibition center; Constantine, Algeria, Oran. \(^c\)Public market ; Maraval, Oran, Algeria. \(^d\)Public market ; Oran, Algeria. \(^e\)Onab, Sidi Bel Abbes, Algeria. \(^f\)Prolabo ; Fontenay-sous-Bois, France. \(^g\)UAR 205 B ; Villeneuvois-. Epinay-sur-Orge; France: mineral mix (mg/kg diet) CaHPO\(_4\), 17200, KCl 4000, NaCl 4000, MgO 420, MgSO\(_4\) 2000, Fe\(_2\)O\(_3\) 120, FeSO\(_4\) - 7H\(_2\)O 200, MnSO\(_4\)-H\(_2\)O-H\(_2\)O 98, CuSO\(_4\) - 5H\(_2\)O 20, ZnSO\(_4\), 80, CuSO\(_4\) 80, CuSO\(_4\) - 7H\(_2\)O 0.32. \(^h\)UAR 200 ; Villeneuvois-, Epinay-sur-Orge, France, vitaminic mix (mg/kg diet): Vit A 39,600 IU, Vit D3 5000 IU, Vit B1 40, Vit B2 30, Vit B3 140, Vit B6 20, VitB7 300, Vit B12 0.1 Vit , 1600; Vit E, 340, Vit K 3.8, Vit PP 200, choline 2720, foie gras acid, 1, para-aminobenzoic acid (PAB) 180, Biotine 0.6.
Analytical methods

Glycemia was measured by strips test (ACCU-CHEK Active, Roche Diagnostics, Mannheim, Germany). Serum total cholesterol (TC), triacylglycerols (TG) and phospholipids (PL) were estimated by enzymatic colorimetric methods (CHOD-POD Spinreact, Sant Esteve d’en Bas, Spain). Liver total lipids were extracted by the method of Delsal [18] as followed: One gram of liver was homogenized with 20 ml of chloroform:methanol (4:1, v:v) and after complete solvents evaporation, the lipid extract was weighed several times, until constant weight. Liver TC, TG (Biocon, Vohl-Marienhagen Germany) and PL (Cypress, Langdorp, Belgium) concentrations were estimated by enzymatic colorimetric methods. Unesterified cholesterol (UC) was evaluated by enzymatic method (CHOD-PAP Biolabo, Maizy, France). In addition, UC/PL ratio as the main membrane fluidity modulator was estimated.

Separation of lipoproteins

Serum VLDL and LDL-HDL3 were isolated by precipitation using MgCl2 and phosphotungstate (Sigma Chemical Company, Lyon, France) by the method of Burstein et al [19]. Serum HDL2 and HDL3 were separated by precipitation according to the method of Burstein et al [20] using MgCl2 and dextran sulfate MW 500,000 Da (Sigma Chemical Company).

Serum HDL2 and HDL3 amounts and compositions

HDL2 and HDL3-TC, -TG, -UC and -PL concentrations were determined by kit methods as mentioned above. HDL2 and HDL3 apolipoprotein (apo) levels were determined by an enzymatic colorimetric method (Chronolab, Barcelona, Spain). Esterified cholesterol (EC) concentrations were obtained by calculating the difference between TC and UC values. Cholesteryl ester (CE) levels were estimated as 1.67 times the TC content. Serum HDL2 and HDL3 amounts which represent total contents of apo’s and lipids (TG, PL, UC and CE) expressed in g/l were estimated.

Assay for LCAT activity

LCAT activity was determined on fresh serum by an endogenous method according to Albers et al [21]. LCAT is the key enzyme responsible for the conversion of UC to EC after 4 h of incubation at 37°C, from a fatty acid and lecithin, in most cases in HDL. LCAT activity is expressed in nmo l/ml/h. LCAT activity is calculated using the following formula:

\[
\text{LCAT activity} = \frac{(\text{UC}_{\text{in}} - \text{UC}_{\text{out}})}{4 \times \text{h incubation}}
\]

Statistical analysis

Data are expressed as means ± SEM for eight rats per group. Statistical analysis was carried out by Student’s t-test (Statistica 6.0 for Windows, StatSoft; Tulsa, OK, USA). P < 0.05 was considered to indicate a significant difference between the both groups: Wheat-lupin and oat-lupin.

RESULTS

Body weight, liver relative weight and food intake

After induction of obesity (day 0), food intake represented 30 g/d compared with the control group (20 g/d) (data not shown) and the obese rats have a BW of 400 ± 10 g. At 28 days of the experiment, body weight, liver relative weight and food intake were similar in the both groups (Table 2). However, compared to day 0, BW value had a tendency to decrease.

Glycemia, serum and liver lipids

At day 0, the obese rats have a cholesterolemia of 2.8 ± 0.6, a triacylglycerolemia of 1.34 ± 0.05 and a glycemia of 11 ± 0.6 mmol/l. At day 28, wheat-lupin and oat-lupin diets significantly decreased hyperglycemia 1.4-fold, hypercholesterolemia 1.6- and 1.4-fold, and hypertriaclyglycerolemia 2.4- and 3.2-fold, respectively, when compared with baseline (day 0) values.

At day 28, glycemia was similar in the wheat-lupin group compared with the oat-lupin group, whereas triacylglycerolemia was significantly enhanced (+25%). Furthermore, cholesterolemia value had a tendency to decrease, but not significantly. On the other hand, liver TG and PL concentrations were enhanced by 46% and 44%, respectively (Table 3).

Lipoproteins lipids concentrations

LDL-C, HDL2-C, HDL3-C and HDL3-C contents were not significantly different in the both groups, whereas serum VLDL-C concentration was decreased by 43% in the wheat-lupin group compared with the oat-lupin group (Table 3).

Table 2. Body weight, liver relative weight and food intake in rats fed experimental diets for 28 days

<table>
<thead>
<tr>
<th></th>
<th>Wheat-Lupin</th>
<th>Oat-Lupin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>386 ± 14</td>
<td>387 ± 17</td>
<td>0.899</td>
</tr>
<tr>
<td>Food intake (g/day/rat)</td>
<td>27.34 ± 5</td>
<td>27.31 ± 5.2</td>
<td>0.991</td>
</tr>
<tr>
<td>Liver relative weight</td>
<td>2.57 ± 0.2</td>
<td>2.63 ± 0.2</td>
<td>0.558</td>
</tr>
</tbody>
</table>

Data were expressed as means ± SEM for eight rats per group. Two groups fed diets containing either 1/3 of white lupin + 2/3 of wheat (wheat-lupin group) or 1/3 of white lupin + 2/3 oat (oat-lupin group). Statistical analysis was carried out by Student’s t-test. Relative weight = (organ weight/body weight) x 100, which provides information of body weight growth compared to the whole organism.
Serum HDL₂ and HDL₃ amounts and compositions
In the wheat-lupin compared to the oat-lupin group, HDL₂ amount, which is the sum of apo’s, TG, CE, UC and PL contents was increased (+46%), whereas HDL₃ amount was comparable. HDL₂-TG concentrations were higher (+52%) (Table 4).

**Statistical analysis was carried out by the Student’s t-test.**

**LCAT activity and atherogenic index**
A significant increase (+31%) in serum LCAT activity was observed in the wheat-lupin group compared with the oat-lupin group. TC/HDL-C, VLDL-LDL-HDL₁₋₃/HDLC and UC/PL atherogenic ratios were similar in the both groups (Table 5).

### Table 3. Glycemia, serum and liver lipids and lipoproteins cholesterol in rats fed experimental diets for 28 days

<table>
<thead>
<tr>
<th></th>
<th>Wheat-Lupin</th>
<th>Oat-Lupin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum lipids (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.65 ± 0.4</td>
<td>2 ± 0.3</td>
<td>0.069</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>0.55 ± 0.09***</td>
<td>0.41 ± 0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.41 ± 0.16*</td>
<td>0.26 ± 0.1</td>
<td>0.041</td>
</tr>
<tr>
<td><strong>Lipoproteins cholesterol (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.4 ± 0.10**</td>
<td>0.71 ± 0.13</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL-HDL₁₋₃-C</td>
<td>0.22 ± 0.01</td>
<td>0.21 ± 0.06</td>
<td>0.649</td>
</tr>
<tr>
<td>HDL₁-C</td>
<td>0.34 ± 0.02</td>
<td>0.33 ± 0.002</td>
<td>0.181</td>
</tr>
<tr>
<td>HDL₂-C</td>
<td>0.72 ± 0.03</td>
<td>0.75 ± 0.01</td>
<td>0.178</td>
</tr>
<tr>
<td><strong>Liver lipids (µmol/g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>16.59 ± 14.8</td>
<td>20.91 ± 8.7</td>
<td>0.488</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>226.06 ± 78.7**</td>
<td>120.13 ± 28.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>76.81 ± 13.2***</td>
<td>43.07 ± 4.68</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Two groups fed diets containing either 1/3 of white lupin + 2/3 of wheat (wheat-lupin group) or 1/3 of white lupin + 2/3 oat (oat-lupin group). Statistical analysis was carried out by the Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001 for wheat-lupin vs oat-lupin.

### Table 4. Serum HDL₂ and HDL₃ amounts and compositions in rats fed experimental diets for 28 days

<table>
<thead>
<tr>
<th></th>
<th>Wheat-Lupin</th>
<th>Oat-Lupin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL₂</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amounts (g/l)</td>
<td>22.5 ± 8.94*</td>
<td>12.03 ± 4.35</td>
<td>0.010</td>
</tr>
<tr>
<td>Apo’s (g/l)</td>
<td>1.65 ± 0.01</td>
<td>1.53 ± 0.09</td>
<td>0.222</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.23 ± 0.001***</td>
<td>0.11 ± 0.002</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PL (mmol/l)</td>
<td>0.01 ± 0.0005</td>
<td>0.01 ± 0.0002</td>
<td>1</td>
</tr>
<tr>
<td>UC (mmol/l)</td>
<td>0.023 ± 0.003</td>
<td>0.021 ± 0.002</td>
<td>0.139</td>
</tr>
<tr>
<td>CE (mmol/l)</td>
<td>0.32 ± 0.001</td>
<td>0.31 ± 0.004</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Two groups fed diets containing either 1/3 of white lupin + 2/3 of wheat (wheat-lupin group) or 1/3 of white lupin + 2/3 oat (oat-lupin group). Statistical analysis was carried out by the Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001 for wheat-lupin vs oat-lupin.

### Table 5. LCAT activity and atherogenic index in rats fed experimental diets for 28 days

<table>
<thead>
<tr>
<th></th>
<th>Wheat-Lupin</th>
<th>Oat-Lupin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LCAT activity (nmol/ml/h)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>2.2 ± 0.5</td>
<td>1.8 ± 0.2</td>
<td>0.054</td>
</tr>
<tr>
<td>VLDL-LDL-HDL₁₋₃-C/HDLC</td>
<td>0.86 ± 0.28</td>
<td>0.85 ± 0.19</td>
<td>0.935</td>
</tr>
<tr>
<td>UC/PL</td>
<td>3.94 ± 0.36</td>
<td>3.95 ± 0.73</td>
<td>0.973</td>
</tr>
</tbody>
</table>

Two groups fed diets containing either 1/3 of white lupin + 2/3 of wheat (wheat-lupin group) or 1/3 of white lupin + 2/3 oat (oat-lupin group). Statistical analysis was carried out by the Student’s t-test. ***P < 0.001 for wheat-lupin vs oat-lupin.
DISCUSSION

The purpose of this study was to investigate the effects of cereals and legumes association on hyperglycemia, dyslipidemia and reverse cholesterol transport in rats fed a high-fat-diet. There is growing research on the relation between the Mediterranean diet (MD) and obesity, with an increasing number of epidemiological studies examining this association [22]. To approach the MD model, we chose in our experiment two associations of whole-grain cereals (2/3 wheat or 2/3 oat) and legume (1/3 white lupin).

Experimental models are necessary in obesity investigation because they allow more controlled experimental conditions, although care must be taken to extrapolate these facts to human subjects. Although some limitations of this study included lack of a control group and small sample size, we demonstrated distinct and favorable effects of legumes and cereals association on obesity in rats. After induction of obesity (day 0), the high-fat-diet induced in all animals an obesity characterized by a weight gain, an increase in food intake, hyperglycemia and dyslipidemia (hypertriacylglycerolemia and hypercholesterolemia).

It is well established that the high density diet decreases satiety and increases body weight [23]. In the present experiment, it appears that the both associations have been well accepted by all obese rats. In spite of a high animal fat consumption (20%), wheat-lupin and oat-lupin diets slightly reduced the body weight of rats at day 28 as compared with day 0. Despite their content of lipids, starch and proteins, legumes are claimed to help maintaining a regular body weight, due to their activity and/or by reducing the NADPH required for cholesterol biosynthesis especially by decreasing the activity which was sufficient to attenuate the hypertriglyceridemia induced with fat feeding. This result could be due to elevated lipoprotein lipase activity which was sufficient to attenuate the hypertriacylglycerolemia induced with fat feeding. The involvement of pulses other than soybean in the control of lipidemic homeostasis has been considered [37]. At day 28, wheat-lupin and oat-lupin diets significantly decreased the hypertriacylglycerolemia and hypercholesterolemia observed at day 0. This result could be due to elevated lipoprotein lipase activity which was sufficient to attenuate the hypertriacylglyceridemia induced with fat feeding. Legumes-cereals interaction may act by decreasing the cholesterol biosynthesis especially by decreasing the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity and/or by reducing the NADPH required for fatty acids and cholesterol synthesis. On the other hand, it can be suggested that wheat-lupin or oat-lupin led to a decrease in cholesterol absorption from the intestine and may have the ability to inhibit the intestinal absorption of bile acids and neutral steroids and to enhance hepatic cholesterol 7a-hydroxylase activity. The decreased levels of serum TC, TG and glycemia in the both groups may be due to the presence of bioactive molecules like saponins, polyphenols, alkaloids and fibers. Several fundamental mechanisms can be proposed to explain our results.

Large population studies demonstrated that plasma levels of HDL-C confer protection against the development of CVD [38, 39]. In our experiment, at day 28, wheat-lupin compared to the oat-lupin group acts slightly on hypercholesterolemia, inversely, it increases triacylglycerolemia. However, HDL_{3-C} and HDL_{2-C} contents were not significantly different in the both groups. In addition, TC/HDL-C and VLDL-LDL-
HDL\textsubscript{2}-C/HDL-C atherogenic ratios as well as UC/PL ratio (the main membrane fluidity modulator) were comparable indicating a similar effect of both diets. The latter result supposed the same UC substitution in favor with PL in cells animal membranes.

Wheat-lupin compared to the oat-lupin association increased serum LCAT activity, despite similar HDL\textsubscript{2}-CE (product of enzymatic reaction), HDL\textsubscript{3}-PL (substrate of LCAT) and HDL\textsubscript{3}-UC (acceptor of lecithin acyl group) contents in the both groups. The enhanced LCAT activity could possibly be related to an increase in its hepatic genes synthesis in this group and consequently counteracts the hyperlipidemic effect.

It is generally accepted that high cholesterol esterification rates promote efflux of cholesterol from peripheral tissues and, therefore, LCAT is able to facilitate the removal of excess cell cholesterol to the extracellular compartment [40]. Hence, it is likely that LCAT promotes the supposedly anti-atherosclerotic RCT pathway, whereby excess peripheral cell cholesterol is delivered to the liver for metabolism and excretion in the bile [41].

Legumes were considered as an ideal complement to cereals [42]. They are an excellent source of many essential nutrients, including proteins, fibers, antioxidants, and other bioactive compounds, as well as being linked with health promoting benefits, such as reducing risk for CVD [42]. It can, therefore, be concluded that these associations may contribute greatly in the management and/or prevention of cardiometabolic risk (excess body weight, hyperinsulinemia, hyperlipidemia, inflammation, and oxidative stress), which are the major cardiovascular risk factors.

To conclude, consuming wheat-lupin or oat-lupin prevent the weight gain, hyperglycemia, hypertriacylglycerolemia and hypercholesterolemia in obese rats. It appears that wheat-lupin has a cholesterol lowering effect and improves RCT by acting efficiently on the efflux of cholesterol from peripheral tissues to the liver by increasing LCAT activity. In obese rats, wheat-lupin association may have a protective effect against cardiovascular risk by improving the anti-atherogenic metabolic pathway of cholesterol.

**ABBREVIATIONS**

BW: body weight  
CVD: cardiovascular disease  
EC: esterified cholesterol  
HDL: high density lipoprotein  
LDL-C: low density lipoprotein-cholesterol  
LCAT: lecithin-cholesterol acyltransferase  
MD: Mediterranean diet  
PL: phospholipids  
RCT: reverse cholesterol transport  
TC: total cholesterol  
TG: triacylglycerols  
UC: unesterified cholesterol  
VLDL: very low density lipoprotein  
VLDL-C: very low density lipoprotein-cholesterol

**ACKNOWLEDGEMENTS**

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**COMPETING INTERESTS**

The authors declare that they have no conflicts of interest to disclose.
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