Microgram amounts of abscisic acid in fruit extracts improve glucose tolerance and reduce insulinemia in rats and in humans

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ABSTRACT 2-Cis,4-trans-abscisic acid (ABA) is a plant hormone that is present also in animals. Several lines of evidence suggest that ABA contributes to the regulation of glycemia in mammals: nanomolar ABA stimulates insulin release from β-pancreatic cells and glucose transporter-4–mediated glucose uptake by myoblasts and adipocytes in vitro; plasma ABA increases in normal human subjects, but not in diabetic patients, after a glucose load for an oral glucose tolerance test (OGTT). The presence of ABA in fruits prompted an exploration of the bioavailability of dietary ABA and the effect of ABA-rich fruit extracts on glucose tolerance. Rats underwent an OGTT, with or without 1 µg/kg ABA, either synthetic or present in a fruit extract. Human volunteers underwent an OGTT or a standard breakfast and lunch, with or without a fruit extract, yielding an ABA dose of 0.85 or 0.5 µg/kg, respectively. Plasma glucose, insulin, and ABA were measured at different time points. Oral ABA at 0.5–1 µg/kg significantly lowered glycemia and insulinemia in rats and in humans. Thus, the glycemia-lowering effect of low-dose ABA in vivo does not depend on an increased insulin release. Low-dose ABA intake may be proposed as an aid to improving glucose tolerance in patients with diabetes who are deficient in or resistant to insulin.—Magnone, M., Ameri, P., Salis, A., Andraghetti, G., Emionite, L., Murialdo, G., De Flora, A., Zocchi, E. Microgram amounts of abscisic acid in fruit extracts improve glucose tolerance and reduce insulinemia in rats and in humans. FASEB J. 29, 4783–4793 (2015). www.fasebj.org

Key Words: plasma glucose · plasma insulin · apricots · OGTT · standard B&L.

2-Cis,4-trans-abscisic acid (ABA) is a plant hormone that regulated important physiologic functions, such as the response to abiotic stress (water and nutrient availability, UV irradiation), seed dormancy and germination, and root elongation (1). It is also present in mammalian plasma (2), and several cell types have been shown to produce it, and cells respond to autocrine or paracrine ABA with the activation of tissue-specific functions. Chemical or physical stimuli induce ABA release from human granulocytes, and ABA in turn stimulates phagocytosis, cell migration, and production of reactive oxygen species (3). Activated platelets or quartz particles stimulate ABA release from monocytes/macrophages and ABA, in an autocrine manner, stimulates release of cytokines and prostaglandin E2 (4, 5). IL-8 induces human mesenchymal stem cells to release ABA, which stimulates the release of growth factors from these cells and colony growth from human hemopoietic progenitors (6, 7). An intriguing clue to the conserved role of ABA as a stress hormone in plants and animals comes from the observation that UV light stimulates NO release in both Arabidopsis and human keratinocytes via ABA production (8, 9).

Several lines of evidence suggest that ABA is involved in the regulation of glucose metabolism in humans. High-glucose concentrations stimulate β-pancreatic cells to release ABA, which in turn stimulates insulin release (10). Plasma ABA (ABAp) concentration increases in humans after a glucose overload, indicating that it is an endogenous hormone (2). In vitro, ABA stimulates glucose uptake in murine cells, independent of insulin, by increasing the expression and membrane translocation of the glutamate transporter (GLUT)-4 (2), the GLUT predominantly expressed in skeletal muscle. Most recently, an impairment of the hyperglycemia-induced increase in ABAp was observed in subjects with type 2 diabetes (T2D) and with gestational diabetes mellitus (GDM). The increase in ABAp in response to glucose is abrogated in patients with T2D compared with normal glucose–tolerant (NGT) controls, and a similar result has been observed in women with GDM compared with pregnant NGT controls. An interesting finding was that 1 mo after childbirth, both fasting

Abbreviations: ABA, 2-cis,4-trans-abscisic acid; ABAp, plasma concentration of ABA; AUC, area under the curve; B&L, breakfast and lunch; GDM, gestational diabetes mellitus; GLUT, glucose transporter; HPLC-MS, HPLC-coupled mass spectrometry; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test; T2D, type 2 diabetes; TFA, trifluoroacetic acid

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ABAp and ABAp response to glucose are restored to normal in subjects with GDM, along with glucose tolerance (11). Finally, fasting ABAp increases significantly 1 mo after biliopancreatic diversion in obese patients with T2D, in parallel with a significant reduction of fasting plasma glucose. Altogether, these in vivo results strongly suggest a beneficial effect of elevated ABAp on glycemic control in humans.

That ABA is present in plants and fruits and that it has biologic functions in animal cells prompted us to explore the bioavailability of dietary ABA and the effect on glycemia of ABA-rich food. To this end, we first identified ABA-rich food sources, obtained ABA-enriched extracts from the foods, and tested their effect on glycemic tolerance in rats and in humans. Regarding the extraction method, water was chosen for the following reasons: an aqueous extract is preferable for human use, as it does not contain potentially toxic solvents; ABA is water soluble at concentrations lower than 25 mM at a neutral pH; and an aqueous extract can be lyophilized and easily reconstituted before consumption.

MATERIALS AND METHODS

Measurement of ABA content in various food sources

Samples (2 g wet weight) of the fruits and vegetables listed in Table 1 were homogenized in 2 ml water and sonicated 3 times at 3 W for 20 s on ice, and 4 vol distilled methanol was added. For determination of the ABA content in the standard breakfast and lunch (B&L), one-tenth of the amount of each component was mixed and homogenized, and 2 g of the homogenate was added to 4 vol distilled methanol. Trace amounts of $\text{[}^2\text{H}]$-ABA (3 x $10^9$ counts per million (cpm)) were added as an internal standard to each sample, and all extracts were kept at $-20^\circ\text{C}$ for 24 h before further processing. After centrifugation (2000 g for 10 min), the supernatants were dried in a Rotovap (Büchi Labortechnik AG, Flawil, Switzerland), the methanol extracts were dissolved in 3 ml deionized water, the pH was adjusted to 2.5 with 10% tri fluoroacetic acid, and the diluted extract was analyzed by HPLC-MS. The amount shown would yield a dose of ABA of 1 $\mu$g/kg in a subject weighing 80 kg.

<table>
<thead>
<tr>
<th>Food</th>
<th>ABA (pmol/g wet weight)</th>
<th>Amount of food (g) containing ~80 $\mu$g ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig</td>
<td>2812</td>
<td>110</td>
</tr>
<tr>
<td>Bilberry</td>
<td>1444</td>
<td>200</td>
</tr>
<tr>
<td>Apricot</td>
<td>1220</td>
<td>250</td>
</tr>
<tr>
<td>Banana</td>
<td>855</td>
<td>364</td>
</tr>
<tr>
<td>Potato</td>
<td>119</td>
<td>2580</td>
</tr>
<tr>
<td>Soy milk</td>
<td>117</td>
<td>2580</td>
</tr>
<tr>
<td>Apple</td>
<td>90</td>
<td>3330</td>
</tr>
<tr>
<td>Olive</td>
<td>62</td>
<td>5000</td>
</tr>
</tbody>
</table>

All food was from Italy, except the bananas, which were from Africa. ABA content in methanol extracts of the various foods was determined by HPLC-MS. The amount shown would yield a dose of ABA of 1 $\mu$g/kg in a subject weighing 80 kg.

Aqueous extraction of apricots and apples

Fresh ripe fruits were cut into small pieces and completely dried at 50°C for 36 h. Dried samples were stored at 4°C in a vacuum. For extraction, samples were weighed and rehydrated for 30 min in a beaker containing ultrapure water (4 ml water/g sample). After rehydration, samples were homogenized and filtered (Whatman filter paper; GE Life Sciences, Little Chalfont, United Kingdom) in a vacuum, both the filtrate and the filter residue were centrifuged at 660 g for 10 min, and the supernatants were recovered and mixed. The amount of ABA in the aqueous extracts was measured by a specific ELISA (Agilia, Elkhart, IN, USA) and confirmed by liquid chromatography (LC)/MS analysis.

Oral glucose tolerance tests in rats

Male Wistar rats (7 wk) were purchased from Charles River (Milan, Italy) and housed at the animal facility of the IRCCS San Martino–IST (Genoa, Italy). The protocol of animal use was approved by the ethics committee of the animal facility, and all procedures involving animals were performed according to European Community directives.

The rats were divided into 3 groups (12 animals/group) and remained unfed for 17 h. After mild sedation with diazepam, 1 g/kg glucose, containing 1 $\mu$g/kg ABA (Sigma-Aldrich, Milan, Italy) or none, was administered by gavage in 300–400 $\mu$l water solution. The animals were then anesthetized with xylazine and ketamine. Blood was drawn from the orbital sinus before gavage (time 0) and 15, 30, 60, and 120 min after gavage. Glycemia was immediately measured with a glucometer, with each measure performed in duplicate. An aliquot of each blood sample was deproteinized with heparin and was immediately centrifuged at 22,000 g for 30 s, and plasma aliquots were stored at $-20^\circ\text{C}$ for the determination of insulinemia: each sample was assayed in duplicate.

Human volunteers

The human study was approved by the San Martino Hospital Ethical Committee (San Martino Hospital, Genoa, Italy) and was

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**TABLE 1. ABA content in some fruits and vegetables common in the Western diet**

<table>
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</tr>
</tbody>
</table>
OGTTs in humans

The OGTTs were performed in the morning, after overnight fasting. Six volunteers underwent a standard OGTT. The 3 subjects who volunteered for the OGTTs, without or with apricot extract, underwent 2 tests each, 1 with glucose alone (OGTT w/o extract; i.e., a standard OGTT), the other with the same amount of glucose supplemented with an amount of aqueous apricot extract yielding a dose of 0.85 µg/kg of ABA (OGTT +extr). The 2 tests were performed 1 wk apart: subjects 5 and 6 underwent the OGTT+extr first, and subject 7 underwent the OGTT w/o extract first. All participants drank 150 ml of a 50% (w/v) glucose solution (Sclavo Diagnostics International, Siena, Italy) in 5 min. Blood sampling was performed immediately before intake of glucose (time 0) and after 30, 60, and 120 min. Blood samples were processed as described below for determination of glucose, insulin, and ABA concentrations.

Experimental B&L

After fasting overnight, 7 volunteers consumed a standard breakfast, followed 4 h later by a standard lunch (see Table 3 for meal composition). Each subject volunteered in 2 experiments, one without (control), the other with an aqueous fruit extract (either from apples, subjects 1–3, or from apricots, subjects 11–14). The amount of extract was calculated to yield a dose of 0.5 µg/kg of ABA and was consumed immediately before breakfast. Subjects 1, 2, 11, and 12 performed the experiment without extract first, and subjects 3, 13, and 14 performed the experiment with the extract first. Immediately before breakfast (time 0) and after 30, 60, 120, 240, and 360 min, blood samples (5 ml) were drawn in heparin and processed as described below for measurement of ABAP, glucose, and insulin concentrations.

Measurement of plasma glucose, insulin, and ABA

Human blood samples (5 ml), drawn in heparin, were centrifuged immediately after withdrawal at 2000 g for 10 min. Two milliliters of plasma was immediately extracted with 4 vol distilled methanol for determination of ABAP. The rest of the plasma and the methanol extracts were stored at −20°C and then processed for glucose, insulin, and ABA measurements. All measurements were made in duplicate and in the same batch when from the same subject. Plasma glucose concentration was measured by an enzymatic method (Randox Laboratories, Crumlin, United Kingdom). Values obtained were always very similar (SD ≤3%) to those measured in capillary blood at the same time point with a glucometer (Bayer, Milan, Italy). Plasma insulin concentration was determined by a sandwich immunoradiometric assay (Immunotech, Prague, Czech Republic), with sensitivity, intra-assay variability, and interassay variability of 0.5 µIU/ml, ≤4.3%, and ≤3.4%, respectively. ABA extraction and determination of its concentration by HPLC-MS were then performed (2).

Rat plasma insulin concentration was determined with an ELISA kit (Bertin-Pharma, Montigny, France).

The areas under the curve (AUCs) of ABAP, glucose, and insulin were calculated with the trapezoidal rule, from the concentrations measured at the time points indicated above, relative to the respective value at time 0.

Statistical analysis

Continuous variables are presented as means ± SD. Comparisons were drawn by unpaired, 2-tailed Student’s t test, if not otherwise indicated. Statistical significance was set at P < 0.05.

TABLE 2. Volunteers participating in the study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>cm × kg</th>
<th>BMI</th>
<th>Experiment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>F</td>
<td>160 × 50</td>
<td>19.5</td>
<td>Intake of fresh apricots</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>F</td>
<td>163 × 62</td>
<td>23.3</td>
<td>B&amp;L with and w/o apple extract</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>M</td>
<td>180 × 74</td>
<td>22.8</td>
<td>Intake of fresh apricots</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>M</td>
<td>187 × 85</td>
<td>24.3</td>
<td>B&amp;L with and w/o apple extract</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>F</td>
<td>158 × 50</td>
<td>20.0</td>
<td>Intake of fresh apricots</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>F</td>
<td>162 × 52</td>
<td>19.8</td>
<td>B&amp;L with and w/o apple extract</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>M</td>
<td>173 × 68</td>
<td>22.7</td>
<td>OGTT with and w/o apricot extract</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>F</td>
<td>168 × 57</td>
<td>20.2</td>
<td>OGTT</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>M</td>
<td>176 × 71</td>
<td>22.9</td>
<td>OGTT</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>M</td>
<td>185 × 77</td>
<td>22.5</td>
<td>OGTT</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>F</td>
<td>160 × 52</td>
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<td>B&amp;L with and w/o apple extract</td>
</tr>
<tr>
<td>12</td>
<td>36</td>
<td>F</td>
<td>165 × 55</td>
<td>20.2</td>
<td>B&amp;L with and w/o apple extract</td>
</tr>
<tr>
<td>13</td>
<td>35</td>
<td>M</td>
<td>174 × 68</td>
<td>22.5</td>
<td>B&amp;L with and w/o apple extract</td>
</tr>
<tr>
<td>14</td>
<td>33</td>
<td>M</td>
<td>180 × 72</td>
<td>22.2</td>
<td>B&amp;L with and w/o apple extract</td>
</tr>
</tbody>
</table>

All volunteers were in good health and none had any dietary restriction or was under medication during the time of the experiments.
ABA-rich fruits include apricots and figs

Several fruits and vegetables common in a Western diet were extracted, and their ABA content was determined by HPLC-MS (Table 1). Among the fruits tested, fresh, ripe figs, bilberries, and apricots showed the highest ABA content per gram of wet weight. Because of their high ABA content and their palatable taste to the volunteers, apricots were chosen for the experiment to assess whether intake of ABA-rich food would induce an increase in ABAp.

Intake of apricots increased ABAp in human subjects to a much higher degree than did glucose overload

After overnight fasting, 4 volunteers (subjects 1–4; Table 2) consumed 135 g each of fresh apricot, containing 165 nmol (43.5 μg) of endogenous ABA, as determined by HPLC-MS analysis. The dose of ABA taken by subjects 1–4 was 0.9, 0.7, 0.6, and 0.5 μg/kg body weight, respectively. Blood samples (5 ml) were drawn in EDTA before (time 0) and 15, 30, 60, and 120 min after the meal, for determination of plasma glucose and ABA concentrations. Glycemia and ABAp were compared with those measured in 12 subjects who underwent an OGTT: 6 were enrolled in the study, and 6 others had volunteered in a previous study (2). Their glycemia and ABAp values were pooled to increase the sample number (Fig. 1). An increase in the ABAp after intake of apricots (which contain carbohydrates) could be anticipated, because intake of glucose induces an increase in ABAp in humans (2). Indeed, ABAp increased in all 4 subjects after intake of apricots, with peak

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**TABLE 1. Composition of the standard B&L Meal**

<table>
<thead>
<tr>
<th>Meal</th>
<th>Amount</th>
<th>ABA content (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain cookies (Oro Saiwa)</td>
<td>96 g</td>
<td>3.4</td>
</tr>
<tr>
<td>Whole milk</td>
<td>175 ml</td>
<td></td>
</tr>
<tr>
<td>Cherry jam</td>
<td>40 g</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti</td>
<td>180 g</td>
<td>0.9</td>
</tr>
<tr>
<td>Olive oil</td>
<td>5 g</td>
<td></td>
</tr>
<tr>
<td>Yogurt</td>
<td>125 g</td>
<td></td>
</tr>
</tbody>
</table>

The endogenous ABA content of the breakfast and the lunch was determined by HPLC-MS on methanol extracts of 2 g per aliquot of the homogenates obtained by mixing one-tenth of the amount indicated for each component.

**RESULTS**

ABA-rich fruits include apricots and figs

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values reaching 54, 42, 15, and 23 times the time 0 values in subjects 1–4, respectively (Fig. 1A). The AUC between 0 and 120 min of ABAP was 3725, 2925, 2147, and 1065, respectively. The mean ABAP levels in the 4 subjects were significantly higher than those measured during the 12 OGTTs (Fig. 1B). In contrast, the mean glycemia profile of the 4 subjects after consumption of apricots was significantly lower than the glycemia profile measured during the OGTTs (Fig. 1O). Thus, compared with a glucose load, intake of apricots resulted in a significantly higher AUC for the ABAP, but a significantly lower AUC for glycemia (Fig. 1D).

The marked elevation of the ABAP after intake of apricots cannot be attributed to the increase in glycemia. A likely explanation for the high ABAP levels after intake of apricots is absorption of the ABAP present in the fruits. Indeed, peak ABAP levels were highest in subjects 1 and 2, who received the highest doses of ABAP per kilogram body weight. If all of the ABAP present in 135 g of apricots (165 nmol) were absorbed and evenly distributed in 50–85 kg body weight, the concentration of ABAP in the blood could be expected to be ~2–3 nM. The peak ABAP levels measured in the 4 subjects fell between 5 and 16 nM (i.e., in that concentration range or even higher), suggesting a high bioavailability of oral ABAP and possibly a long blood half-life of the hormone.

ABA at 1 μg/kg lowered glycemia during OGTT in rats without increasing insulinemia

Altogether, the results described so far suggest that intake of ABAP at a dose between 0.5 and 1 μg/kg contributes to significantly increased ABAP in humans. These results moved us to explore whether a similar dose of ABAP present in an aqueous apricot extract is sufficient to lower glycemia in rats subjected to an OGTT. Dietary intake of ABAP has been reported to improve glucose tolerance in db/db mice fed a high-fat diet (13), but at a dose 3 orders of magnitude higher (100 mg/kg) and in animals with a mutation in the leptin receptor.

Three groups of male Wistar rats (12 per group) were subjected to an OGTT: 1 group received glucose only (control); another group was treated with glucose and an aqueous apricot extract, yielding a dose of ABAP of 1 μg/kg; and a third group was treated with glucose and synthetic ABAP, at a dose of 1 μg/kg. (A fourth group is described later.) The animals receiving the apricot extract, and those treated with synthetic ABAP showed a significantly lower glycemia profile than that of the controls (Fig. 2A) and, consequently, a significant reduction in the AUC for glycemia (Fig. 2B). In contrast, no significant difference in the glycemia profile or the glycemia AUC was observed between the group treated with the ABAP-containing extract and the group treated with synthetic ABAP. In both ABAP-treated groups, a reduction in glycemia was already evident in the 0–30 min time frame (Fig. 2A), indicating the rapid action of oral ABAP.

The AUCs of insulinemia of the rats treated with synthetic ABAP or with the apricot extract were also significantly lower than those of the controls. That both the glycemia and the insulinemia profiles were not significantly different in the rats treated with the apricot extract containing ABAP at 1 μg/kg body weight or with synthetic ABAP at the same dose, suggests that the active molecule present in the extract and responsible for the observed metabolic effects is indeed ABAP.

The sparing effect on insulinemia of ABAP at 1 μg/kg was surprising, given that ABAP stimulates insulin release from human β-pancreatic cells and from rat insulinoma cells in vitro (10). To investigate whether a higher dose of ABAP would have the same sparing effect on insulinemia as the low dose, a fourth group of 6 rats was subjected to an OGTT with synthetic ABAP at 100 mg/kg (Fig. 2A). At that dose, ABAP did not significantly reduce insulinemia compared with that in untreated controls (Fig. 2C), although it reduced glycemia (Fig. 2B).

Intake of an apricot extract yielding a dose of ABAP of ~1 μg/kg lowered glycemia and insulinemia during OGTT in 3 human subjects

Results obtained in the rats prompted us to perform a similar OGTT in humans, to verify whether intake of an aqueous apricot extract, yielding a dose of ABAP of 0.85 μg/kg body weight, could lower glycemia during an OGTT. This dose of ABAP is similar to that received by subject 1 in 135 g fresh apricots (0.9 μg/kg body weight), which is capable of inducing a 50-fold increase in ABAP (Fig. 1). Three subjects volunteered for the OGTTs without or with apricot extract; 3 more were enrolled in as many standard OGTTs, serving as controls (Table 2). To increase the number of control subjects, we included results from 6 OGTTs performed in a previous study (2).

The mean glycemia profile during the OGTT with the apricot extract was significantly lower at the 30 and 60 min time points, compared with the mean values obtained from 12 healthy subjects who underwent a standard OGTT (Fig. 3A). The mean plasma glucose concentration during the OGTT w/o extract in the 3 volunteers was very similar to that measured in the 12 subjects who underwent a standard OGTT.

In all 3 subjects who underwent the OGTT, both without and with the apricot extract, the AUC of glycemia over the 0–120 min time frame was lower in the presence of the extract (AUC with vs. w/o extract, 31.2 vs. 36.4, 8.1 vs. 19.0, and 27.8 vs. 40 in subjects 5, 6 and 7, respectively; P = 0.048, by paired Student’s t test; Fig. 3B). Moreover, the mean glycemia AUC in the 3 OGTTs with extract was significantly lower than the mean AUC in the 12 OGTTs without extract.

In the same 3 subjects, the AUC of ABAP was higher during the OGTT+extr compared with the OGTT w/o extract (Fig. 3C). The absence of significance by paired Student’s t test between AUCs with and without extract is due to the large variation between subjects. However, the mean AUC of ABAP in subjects 5–7 in the experiment with the extract was significantly higher than the value measured in the 12 subjects who underwent a standard OGTT (Fig. 3C). The mean AUC (0–120 min) of ABAP in subjects 5–7 after intake of the extract (Fig. 3C) was on the same order of magnitude as the AUCs of the ABAP in subjects 1–4 after intake of apricots. Thus, an AUC (0–120 min) of ABAP between 1000 and 4000 was associated with a significant reduction in both glycemia and
insulinemia and can be obtained by intake of a dose of ABA ranging between 0.5 and 0.9 mg/kg.

Finally, the AUC of insulinemia in the same subject was lower in the OGTT + extr, compared with that found in the OGTT w/o extract (Fig. 3D; P = 0.049 by paired Student’s t test), and the mean AUC of insulinemia was significantly lower in the 3 subjects during the OGTT + extr compared with that measured in the 12 subjects who underwent a standard OGTT (Fig. 2C), Thus, the improved glucose tolerance in the OGTT with the extract cannot be attributed to a higher release of insulin.

Together, these results indicate that intake of an apricot extract containing ABA at a dose of approximately 1 mg/kg body weight reduced the AUC of glycemia in 3 subjects without increasing insulinemia and that exogenous ABA was absorbed and contributed to the improved disposal of the glucose load.

**Intake of an aqueous fruit extract containing ABA lowered glycemia and insulinemia during a standard meal in 7 subjects**

To verify the effect of exogenous ABA on a more physiological carbohydrate intake, a total of 7 subjects volunteered to eat a standardized B&L, without (B&L w/o extr) or with (B&L + extr) an aqueous fruit extract (from either apricots or apples) containing an amount of ABA (30–40 μg, depending on the volume of the extract taken by each subject) yielding a dose of 0.5 μg/kg body weight: 4 subjects consumed the apricot extract and 3 the apple.
Figure 3. Intake of an aqueous apricot extract providing a dose of ABA of ~1 μg/kg body weight lowered glycemia during an OGTT in 3 human subjects. Three healthy volunteers (subjects 5–7; Table 2) underwent 2 different OGTTs each, 1 without (OGTT no extr) and 1 with an apricot extract (OGTT+extr), yielding a dose of 0.85 μg ABA/kg body weight. Plasma ABA, glucose, and insulin concentrations were measured on blood samples taken 15 min before (time 0), and 30, 60, and 120 min after intake of glucose. Values measured in the 3 subjects during the OGTT+extr are also compared with those obtained during standard OGTTs in 12 subjects, comprising subjects 5–10 (Table 2), plus 6 subjects from a previous study (2). A) Mean glycemia profile in subjects 5–7 after glucose intake without (OGTT no extr) or with (OGTT+extr) extract, compared with the mean glycemia profile from 12 subjects who underwent a standard OGTT. B) AUC of glycemia (0–120 min) in subjects 5–7 in OGTTs without or with extract. The last 2 columns indicate the means ± s.d. of the AUCs of glycemia during OGTTs with (n = 3) or without (n = 12) extract. AUC (0–120 min) of ABAp (C) and insulinemia (D) in subjects 5–7 during OGTTs without or with extract. Means ± s.d. of the AUCs during the OGTTs with extract (n = 3) compared with the corresponding mean AUCs during standard OGTTs (n = 12). Probabilities by unpaired Student’s t test.

extract. The composition of the standard meals is shown in Table 3. The content of ABA present in the food was ~4 μg, and the dose of ABA in the B&L+extr was therefore ~1 log higher than that in the B&L w/o extract. Glycemia, insulinemia, and ABAp were measured on blood samples taken before breakfast (time 0) and 2 and 4 h after breakfast (the latter time point being immediately before lunch), and 2 h after lunch (Fig. 4). A reduction in the AUC of glycemia over the 6 h time frame of the experiment was observed in all subjects during the B&L+extr compared with the B&L w/o extr, regardless of the fruit source of ABA (Fig. 4A, D). This result indicates that the lowering effect of ABA on glycemia spanned at least 6 h after intake of the hormone. Indeed, the AUC of the ABAp was higher in all subjects after intake of the fruit extract, as compared with the B&L w/o extr (Fig. 4B, E), at all time frames, up to 4–6 h after ABA intake. AUCs of insulinemia calculated over the 0–6 h time frame were conversely lower in the B&L+extr than in the B&L w/o extr in all subjects (Fig. 4C, F). Although the number of subjects treated with each extract was small, the AUCs (0–360 min) of glycemia, ABAp, and insulinemia were all significantly different in the experiment with the extract compared with levels in the same experiment without extract (P ≤ 0.05 by paired Student’s t test) (Fig. 5A).

That both fruit extracts, providing a similar dose of ABA, were effective at lowering glycemia and insulinemia and induced a similar increase in the AUC of ABAp suggests that the active ingredient responsible for the observed effects was indeed ABA.

The similarity of the results obtained with the apricot and the apple extracts suggested that we compare the AUCs from the B&L+extr from all 7 subjects with the corresponding AUCs of the B&L w/o extr (Fig. 5). Indeed, a statistically significant decrease in the AUCs of glycemia (Fig. 5B) and insulinemia (Fig. 5D) and a significant increase in the AUCs of ABAp (Fig. 5C) were observed in the B&L+extr vs. the B&L w/o extr.

DISCUSSION

Plant-derived bioactive compounds have been used for the prevention or treatment of human diseases since the earliest civilizations. Some of the pharmacologically most active molecules used to this day were indeed first identified
in plants. These bioactive molecules are usually present at very low concentrations in the plant tissues, requiring their concentration or chemical synthesis to obtain pharmaceutically active doses.

There are currently few commercially available vegetal or plant-derived compositions that target one of the most prevalent metabolic diseases in the industrialized world, T2D. T2D is classified by the World Health Organization as a global epidemic, with 280 million subjects affected worldwide and a constantly growing incidence in developing countries, caused by the improvement of dietary standards and the resultant increased glycemic load.
glycemia, with a net saving of insulin in conjunction with endogenous ABA caused a reduction of insulinemia along with increased insulin secretion. On the contrary, intake of exogenous ABA in humans, the improved glucose tolerance was not caused by standard B&L. We were interested to see that, in both rats and humans, both during an OGTT and after intake of a standard B&L, ABA at 100 mg/kg improves glucose tolerance in vivo, rendered diabetic by mutation of the leptin receptor and a high-fat diet (13). ABA at 100 mg/kg improves glucose tolerance in mice in vivo, mutations (14).

The results described herein indicate that the intake of ABA at a dose of ~1 μg/kg (i.e., several logs lower than previously tested in vitro) lowered glycemia in rats subjected to an OGTT and also improved glucose tolerance in humans, both during an OGTT and after intake of a standard B&L. We were interested to see that, in both rats and humans, the improved glucose tolerance was not caused by increased insulin secretion. On the contrary, intake of exogenous ABA caused a reduction of insulinemia along with glycemia, with a net saving of insulin in conjunction with improved glucose management. This effect of ABA in vivo, which could not be anticipated from the effects of ABA on isolated β-pancreatic and muscle cells in vitro, may be the result of different effects of ABA on these cell types, with stimulation of glucose transport preceding in time and exceeding in extent the stimulation of insulin release. In contrast, human GLUT4-expressing cells may be more sensitive to the effect of ABA than are β-pancreatic cells in vivo. In vitro, human β-pancreatic cells and rat myoblasts were both stimulated by nanomolar ABA to increase insulin release and glucose uptake, respectively (2, 10), although the lowest effective concentrations of ABA were 1.0 and 0.1 nM, respectively, possibly indicating a higher sensitivity of myoblasts to ABA.

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Recent unpublished results indicate that, at 50 mg/kg, oral ABA alone (without glucose) increases insulinemia and reduces glycemia in rats (Bruzzone, unpublished results). This observation indicates that a very high dose of ABA alone, without glucose, can stimulate insulin release. The results shown in Fig. 2C indicate that rats receiving ABA at 100 mg/kg, together with a high glucose load, did not have a reduction in insulinemia compared with that in
the control rats receiving glucose only. Together, these results indicate that, at a very high dose, ABA stimulates both glucose uptake and insulin release, resulting in insulinemia levels higher than those observed with low-dose ABA (1 μg/kg), where stimulation of insulin release did not occur.

Altogether, these results suggest that the main target of low-dose ABA in vivo is glucose uptake.

The mechanism underlying the hypoglycemic action of ABA in vivo at a dose of 1 μg/kg body weight does not depend on the stimulation of insulin release. At this dose, however, it is apparent that the stimulatory effect of ABA on glucose transport prevails over the stimulation of insulin release. Indeed, in rats subjected to an OGTT, ABA at 100 mg/kg did not significantly reduce insulin secretion compared with the controls and with animals treated with ABA at 1 μg/kg (Fig. 2C), perhaps because of the concomitant stimulation by high-dose ABA of glucose uptake and of insulin release, resulting in insulinemia levels higher than those reactive only to hyperglycemia. If this is the case, one could expect a faster decrease in glycemia with 100 mg/kg compared with 1 μg/kg ABA. Indeed, the mean AUC of glycemia in the 0–60 min time frame was lower in rats treated with the high compared with the low dose of the hormone (30.1 ± 5.9 vs. 38.1 ± 6.9, respectively; P = 0.037).

A consensus has been building within the scientific community that the prolonged stimulation of β-cells to release insulin under conditions of chronic hyperglycemia contributes to the eventual demise of the β-cell population (18). For this reason, antidiabetic drugs capable of lowering glycemia without increasing insulinemia are highly desirable. That the lowering of blood glucose by low-dose ABA is not due to an increased insulin release may prove advantageous, if dietary low-dose ABA intake were to be proposed as an aid to improving glucose tolerance in diabetic or prediabetic subjects who are deficient in or resistant to insulin. By reducing the chronic stimulation by hyperglycemia of β-cells to release insulin, prolonged administration of low-dose ABA may support the survival and function of these cells. This hypothesis warrants further investigation.

An AUC of ABAp associated with a significant reduction in glycemia and insulinemia after intake of carbohydrates can be obtained with a dose of ABA ranging between 0.5 and 0.9 μg/kg. This dose range is attainable with a helping of several ABA-rich fruits and vegetables, such as apricots (Table 1). The remarkable increase of the ABAp after intake of apricots (~30-fold over basal values) is much higher than that induced by a glucose load (Fig. 1B) and strongly suggests that fruit-derived ABA is readily absorbed. The bioavailability of ingested ABA implies that intake of ABA-rich food helps control glycemia by providing exogenous ABA, which can potentiate or replace the endogenous hormone. The bioavailability of ingested ABA and the sensitivity to the metabolic effects of the hormone showed some variability among the subjects. In the B&L experiments, subjects 11 and 14 appeared to be early responders, as the major difference between the AUCs of glycemia in the presence or absence of ABA-containing fruit extract was observed after breakfast, in the 0–2 h time frame (Fig. 4A). In contrast, subjects 12, 13, and 2 were late responders, the major difference between the AUCs of glycemia in the presence or absence of ABA-containing extract being observed after lunch, in the 4–6 h time frame (Fig. 4B). In any case, the AUC of glycemia after lunch was lower in all subjects; indeed, despite the interindividual variation, the difference between the mean AUC of glycemia in the B&L + ABA vs. the B&L w/o ABA was statistically significant (P = 0.04; Fig. 5).

Thus, intake of 0.5 μg/kg ABA before breakfast improved glucose tolerance until after lunch in all 7 volunteers. Persistence of the beneficial effect of ABA on glucose disposal for several hours may be due to the slow clearance of the hormone, which binds to plasma proteins (unpublished results) and thus probably escapes rapid renal filtration, which could be expected, given its low molecular mass (264.3 Da). Indeed, in all subjects, the AUC of ABAp after intake of ABA was higher in the 240–360 min time frame than that in the experiment without ABA (Fig. 4B, E).

Guri et al. (13) observed a glycemia-lowering effect of dietary ABA on leptin-deficient mice rendered diabetic with a high-fat diet, although at a dose of ABA (100 mg/kg) several logs higher than that reported herein. This difference suggests that the genetic mutation of the leptin receptor in this mouse strain and the high-fat diet diminished the effectiveness of ABA. However, ABA increases the expression of leptin in human and murine adipocytes (unpublished results). The hypothesis that a functional leptin receptor is essential for the glycemia-lowering effect of ABA requires further study.

Another question that should be investigated further is the possibility that ABA at a dose of approximately 1 μg/kg lacks the proinflammatory effects that are observed in vitro at low micromolar hormone concentrations (3–5). The stimulation by micromolar ABA of the activation of innate immune cells observed in vitro with 1–10 μM ABA (3–5) may not occur in vivo after administration of microgram amounts of ABA, resulting in ABAp levels on the order of tens of nanomoles per liter. The in vitro effects of ABA on human granulocyte phagocytosis, NO production, and migration are markedly dose dependent, with a more than 4-fold increase in the functional response when the ABA concentration is increased from 50 nM to 20 μM (3). This finding suggests that overt proinflammatory effects of ABA in vivo are elicited at concentrations in the micromolar, but not in the nanomolar, range. In contrast, the in vitro effect of ABA on glucose transport on L6 myoblasts in vitro is not dose dependent, with a similar stimulatory effect observed at concentrations ranging from 1 nM to 10 μM (2) and a threshold concentration of 0.1 nM (Bruzzone, unpublished results). The different dose response to ABA of granulocyte activation vs. myoblast glucose uptake suggests that the latter is the functional effect most likely stimulated in vivo at low nanomolar ABA concentrations.

This finding would remove the possibility of an unoward effect of the hormone in subjects with T2D or pre-T2D, conditions known to be sustained by a smoldering inflammmation of the adipose tissue (19–21).

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