Tools for the identification of bioactives impacting the metabolic syndrome: screening of a botanical extract library using subcutaneous and visceral human adipose-derived stem cell-based assays☆

Benjamin M. Buehrera, David J. Duffina, Y. Renee Lea-Curriea, David Ribnickyb, Ilya Raskinb, Jacqueline M. Stephensc, William T. Cefalud, Jeffrey M. Gimblee

aZen-Bio, Inc., Research Triangle Park, NC 27709, USA
bDepartment of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ 08901, USA
cDepartment of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA
dDiabetes and Nutrition Research Laboratories, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA
eStem Cell Biology Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA

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Abstract

Plant extracts continue to represent an untapped source of renewable therapeutic compounds for the treatment and prevention of illnesses including chronic metabolic disorders. With the increase in worldwide obesity and its related morbidities, the need for identifying safe and effective treatments is also rising. As such, use of primary human adipose-derived stem cells represents a physiologically relevant cell system to screen for bioactive agents in the prevention and treatment of obesity and its related complications. By using these cells in a primary screen, the risk and cost of identifying artifacts due to interspecies variation and immortalized cell lines is eliminated. We demonstrate that these cells can be formatted into 384-well high throughput screens to rapidly identify botanical extracts that affect lipogenesis and lipolysis. Additionally, counterscreening with human primary stem cells from distinct adipose depots can be routinely performed to identify tissue specific responses. In our study, over 500 botanical extracts were screened and 16 (2.7%) were found to affect lipogenesis and 4 (0.7%) affected lipolysis.

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1. Introduction

The prevalence of obesity, metabolic syndrome, diabetes and their associated comorbidities has increased significantly across the world over the recent past. As such, it is imperative that we implement successful and viable strategies in order to address the global epidemic. In this regard, and in an historical perspective, extracts from plants, i.e., botanicals, have proven to be a rich resource for the discovery of therapeutic compounds and the sources of many current medicinal drugs [1,2]. Throughout the world, traditional cultures have identified specific plant species for the treatment of multiple disorders, including the metabolic syndrome and diabetes [1]. In particular, plant extracts proposed to contain effective “bioactives” are marketed routinely as therapeutic agents for metabolic syndrome and diabetes, often with limited understanding of mechanism or proof of efficacy. Recently, the World Health Organization and the NIH have supported international efforts to develop comprehensive libraries of botanical extracts for therapeutic drug discovery. Studies have begun to screen these libraries for candidate molecules directed toward the prevention and treatment of the metabolic syndrome using cell-based in vitro assays [3–9].

Given the importance of insulin resistance in peripheral tissues such as adipose tissue and muscle in the etiology of metabolic syndrome and progression to type 2 diabetes, both adipocyte and skeletal muscle cell lines have been employed in these studies [4,8]. In most analyses of adipocytes, investigators have chosen to use the 3T3-L1 murine preadipocyte cell line [4,10–15]. This in vitro model of adipogenesis is characterized by a homogeneous morphology and a robust lipogenic and lipolytic response to known agonists and antagonists [12–15]. However, since the 3T3-L1 cell line is of murine origin, its utility as a drug discovery tool for eventual human conditions has been questioned due to interspecies variations. A

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⁎ Corresponding author. Botanical Research Center, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA.

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number of laboratories, including those in the pharmaceutical industry, have begun to employ primary human adipose-derived stromal/stem cells (ASCs) as an alternative in vitro model for adipocyte differentiation and function [16–23]. The ASC can be derived from many adipose depots in human subjects, including subcutaneous and omental/visceral tissue. There is a growing body of literature indicating that the metabolic function and drug response of adipocytes in these depots are distinct. This has potential implications with respect to the pathology, diagnosis and treatment of metabolic syndrome and diabetes for the human condition [24,25]. The current study reports the use of both primary human subcutaneous- and visceral-derived ASCs to screen a library of botanical extracts for both lipogenic and lipolytic agonists and antagonists. These preliminary studies have the potential to yield novel pharmaceuticals of plant origin for the treatment or prevention of metabolic syndrome.

2. Materials and methods

2.1. Chemicals

2.1.1. Botanical extract library

The source of plant extracts was prepared and obtained from the John S. McIlhenny Laboratory of Botanical Research as part of the NIH-funded Center for the study of Botanicals and Metabolic Syndrome. The extracts were originally obtained as part of the International Cooperative Biodiversity Group program. Field-collected plant samples were prepared by air drying and extraction with 80% ethanol (1.5 mL) three times, infused each time for 24 h and evaporated in a rotary evaporator. The obtained extracts were dried under vacuum and stored in amber glass vials at −20°C. The yield of air-dried extract as the percent weight of the dried plant tissue varied among the tested samples based on the plant part used. Before tests, concentrations were dissolved by sonication in DMSO for a final concentration of 20 mg/mL.

2.1.2. Chemicals and reagents

Type I collagenase was obtained from Worthington Biochemical (Lakewood, NJ). Bovine serum albumin (BSA), dexamethasone, biotin, panethothenate, isoproterenol and isobutyl methylycathine were purchased from Sigma (St. Louis, MO). Human recombiant insulin was obtained from PM Biomedicals (SOLON, OH). The fetal bovine serum, Dulbeco modified Eagle’s medium, phosphate-buffered saline and Ham’s F-12 nutrient broth were from Zen-Bio (RTP, NC). Tumor necrosis factor α was from Roche Diagnostics (Indianapolis, IN). All tissue culture flasks and plates were obtained from Corning (Corning, NY).

2.2. Preparation and culture of primary ASC from subcutaneous and visceral depots

2.2.1. Isolation of human primary ASCs from subcutaneous and omental depots

Human adipose tissue samples were procured from consenting donors undergoing elective surgeries under IRB-approved protocols. Subcutaneous and omental adipose tissue was from surgical waste material derived from subcutaneous liposarpiate or omentum tissue. Human ASCs were isolated from liposarpiate or omental waste tissue as previously described [26,27]. Adipose tissue was extensively washed with PBS prior to processing. Omental tissue samples were hand-minced while washed lipoaspirate material was directly transfused for dissatisfaction with an equal volume of Krebs Ringer buffer containing 0.1% collagenase and 1% BSA. Following a 15- to 45-min incubation at 37°C, floating primary adipocytes and most of the collagenase buffer were removed and PBS was added. The remaining stromal vascular cell suspension was subjected to centrifugation for 5 min at 300 g and the resulting cell pellet washed several times with PBS. Finally, the cell pellet was suspended in preadipocyte medium (PM-1, Zen-Bio) and plated in a culture flask for expansion. ASCs were subcultured prior to reaching confluence and plated at a density of 1.4 million cells per T-225 flask (6000 cells/cm²) for further expansion in PM-1 containing epidermal growth factor and fibroblast growth factor.

2.2.2. Preparation of subcutaneous and omental ASC super-lots

Mixed patient lots of either subcutaneous or omental ASCs were prepared from cryopreserved ASCs derived from four to six donor lots each. The subcutaneous super-lot was derived from six female donors with an average age of 40±3.8 (S.E.) and average BMI of 27.9±0.6. The omental super-lot was derived from four female donors with an average age of 40±3.8 (S.E.) and average BMI of 45.6±4.9. Equal numbers of Passage 1 ASCs were combined in preadipocyte medium and plated for expansion at a density of 2.2 million cells per chamber (3500 cells/cm²) in CellSTACK chambers (Corning). The super-lot cells were expanded to near confluence and harvested by trypsinization for cryopreservation and later use.

To ensure the quality of the prepared cell super-lots, both the omental and subcutaneous lots were tested for differentiation and lipolytic response. Both lots met the required cutoff values for triglyceride accumulation and free fatty acid (FFA) release under standard differentiation procedures. Cell surface markers for both lots of ASCs were confirmed using flow cytometry and antibodies to positive markers (CD29, CD44, and CD105) and negative markers (CD14, CD31, and CD45).

2.3. Screen botanical extracts for human adipocyte lipolysis

Mature adipocytes in clear bottom 384-well plates were prepared by seeding ASCs at a density of 6000 cells/well and differentiating them to adipocytes by adding differentiation medium (DM-2, Zen-Bio) for 7 days. After differentiation, the medium was partially replaced with adipocyte medium (AM-1, Zen-Bio) and the cells incubated for a further 7 days at 37°C with 5% CO₂. This procedure results in greater than 80% adipocyte differentiation [28]. Medium was aspirated and the cells washed twice with 50 μL PBS using a cell washer (ELX-405 Select CW, BioTek) prior to adding 25 μL of assay buffer to each well. Botanical extracts were diluted in a two-step process by using a Matrix PlateMate 2×3 (ThermoScientific); first a 50-fold dilution into assay buffer was performed, followed by a similar 10-fold dilution. Twenty-five microliters of the 500-fold dilution were added to the assay plates in quadruplicate for a final dilution of 1000-fold (20 μg/mL). Isoproterenol (100 nM) and DMSO (0.1%) were added to quadruplicate wells on every plate and served as the positive and negative controls, respectively. Cells and extracts were incubated for 4 h at 37°C with 5% CO₂ to accumulate FFA in the conditioned assay buffer.

For follow-up experiments in 96-well plates, preadipocytes were seeded at a density of 1000 cells/well and differentiated as described above. Cells were washed twice with PBS and treated with extracts in a final volume of 100 μL of assay buffer for 4 h.

2.3.1. Measuring fatty acid release in human cultured adipocytes

FFAs released into the conditioned assay buffer were quantified using Lipolysis Assay Kit reagents (Zen-Bio, Inc.). For the initial screen, 15 μL of conditioned assay buffer was removed from each well and transferred to new 384-well plate for determination of fatty acid release. Fifty microliters of FFA Solution A was added to 15 μL of conditioned assay buffer and incubated at 37°C for 10 min. Twenty-five microliters of FFA Solution B was added to each well and incubated for an additional 10 min at 37°C. Total FFAs released were determined by measuring the optical density at 540 nm and comparison to a standard curve ranging from 1.5 to 333 μM. Quadruplicate values were averaged, and standard deviations were used to determine statistical differences between controls. Follow-up assays were performed in 96-well plates essentially as described but with 30 μL of conditioned assay buffer, 100 μL FFA Solution A and 50 μL FFA Solution B.

To determine if positive results were found from extract interference with the assay reagents, we performed fatty acid assays using extracts diluted into assay buffer without exposure to cells. If the resulting value was above 10 μM, it was concluded that the extract interfered with the assay.

2.4. Screen botanical extracts for lipogenic effects in human adipocytes

ASCs were seeded at 6000 cells/well in clear bottom 384-well plates using PM-1. The next day, a 40% mixture of DM-2 and PM-1 was added and incubated for 7 days at 37°C with 5% CO₂. Four wells were given 100% DM-2 to serve as a positive control. An additional four wells were given PM-1 to serve as an uninduced control. Botanical extracts were added on Day 7 through a two-step dilution process in AM-1 to give a 50 μg/mL final concentration. 0.25% DMSO was added to four wells to serve as a vehicle control and 10 ng/ml TNFα served as a positive control of inhibition. The cells and extracts were incubated at 37°C with 5% CO₂ for an additional 7 days prior to quantifying total accumulated triglyceride.

2.4.1. Triglyceride measurement in subcutaneous and omental adipocytes

The total accumulated triglyceride was determined using Total Triglyceride Kit reagents (Zen-Bio, Inc.). The medium was aspirated and the cells were washed once with 50 μL wash buffer using an automated cell washer (BioTek). Five microliters of lysis buffer was added to each well and cells were incubated at 37°C for 20 min to complete the cellular lysis. Forty-five microliters of wash buffer was added to each well before adding 6.7 μL Reagent B (lipase) and incubating for an additional 2 h at 37°C. 12.5 μL of each lipase treated sample was transferred to a new 384-well plate containing 125 μL of FFA Solution A. A positive control volume of Glycerol Reagent (12.5 μL) was added to each well and incubated for 15 min at room temperature. Glycerol content was determined by measuring the optical density at 540 nm and comparing the values to a glycerol standard curve. There is a 1:1 molar ratio between the amount of glycerol detected and cellular triglyceride content.

For the primary screen, treatments and assays performed in 96-well plates followed a similar protocol, but the volumes were adjusted for the larger plate format. Preadipocytes were seeded at 13,000 cells well prior to differentiation and extract treatment in 150 μL final volume. Volumes were changed to 135 μL lysis buffer, 135 μL wash buffer and 20 μL of Reagent B. Ten microliters of each sample was diluted into 40 μL of wash buffer and 50 μL of Reagent A added to initiate quantification of glycerol.

2.5. Cytotoxicity analysis of botanical extracts

Extract-induced cytotoxicity was assessed in the 384-well format using conditions for the lipogenic assays. Instead of performing the tryglceride assay, 25 μL of
conditioned medium was removed and 5 μl of Cell Titer Blue reagent (Promega) added to each well. Cells and reagent were incubated at 37°C in a humidified 5% CO2 incubator for 2 h. The absorbance at 570 nm was measured for each well and that from the reference wavelength (600 nm) subtracted. The background value (OD_{570−OD_{600}}) from wells without cells was subtracted from each value to determine the extract-induced change in absorbance. Cytotoxicity was reflected by a decrease in the OD_{570} value. 0.1% Triton X-100 served as a positive control for cytotoxicity.

2.6. Statistics

For the screen results, quadruplicate values were averaged and the standard deviation for each treatment determined. Values are displayed based on total cell number seeded per well. Significant differences from control values were determined using a paired Student’s t test with P≤0.05 cutoff. Cutoff values for an active extract identified in the primary screen were established based on being more than 3 standard deviations from the control mean. The screening statistic, Z′, was determined for each assay according to Zhang et al. [29] based on the means and standard deviations of the positive and negative controls using the following equation:

\[
Z' = 1 - \frac{3\times(\text{SD}_1 + \text{SD}_2)}{|\mu_1 - \mu_2|}
\]

where SD1 is positive control standard deviation; SD2 is negative control standard deviation; μ1 is positive control mean; μ2 is negative control mean. EC_{50} and IC_{50} values were determined by fitting dose response data to a four-parameter logistic equation using GraphPad Prism software (GraphPad Software, Inc.).

3. Results

A collection of 580 botanical extracts derived from Central Asian plants were tested for their effects on human adipocyte lipolysis and lipogenesis following the outline depicted in Fig. 1. The extracts are unfractionated botanical mixtures and will serve as starting points to identify active components. The complexity of the mixtures dictated the use of a maximum concentration allowed by the assays to identify active extracts. High throughput primary human cell screening assays were developed for lipolysis and lipogenesis to identify both inhibitors and inducers in a single screen.

3.1. Identification of botanical extracts with lipolytic inductive and suppressive properties in subcutaneous adipocytes

All botanical extracts were examined for their acute lipolytic effect on human subcutaneous adipocytes in culture. To maximize signal to background and minimize the effect of FFA reuptake [30], the cells were exposed to the extracts for 4 h prior to measuring FFA release into the assay medium. The extract concentration was limited to 20 μg/ml to keep DMSO at or below 0.1% in the assay. Fig. 2 shows the effects of each extract on FFA release as percent of the unstimulated control (0.1% DMSO). Most of the extracts had little effect, clustering around the vehicle control (100%). However, 15 extracts were chosen for follow-up studies because their activity was significantly above the screening cutoff value (3 standard deviations above the mean). The Z′ value (see Methods) for this screen was 0.503, an acceptable value for initial screening [29,31].

3.2. Partial lipolysis screen of botanical extracts in human omental cultured adipocytes

OM adiposity is linked to diabetes and metabolic disorders, suggesting that a screen using primary human omental adipocytes should be the most physiologically relevant system to identify novel actives [24,25,32,33]. To determine the utility and overlap of a 384-well-based omental adipocyte screen, half of the extracts were screened for their acute lipolytic effects using human omental adipocytes. After 4 h of extract exposure, the amount of FFA released in the culture medium was determined. A similar number of active extracts were found using omental adipose-derived cells as shown in Fig. 3. Considerable overlap of active extracts was found in the omental screen and further characterization of the primary screen active extracts was performed. The Z′ value for the omental screen...
was determined to be 0.514, on par with the primary subcutaneous adipocyte screen.

### 3.3. Confirmation and dose dependence of lipolytic extracts in both subcutaneous and omental cells

Fifteen extracts (2.6%) from the subcutaneous cell and eight extracts (2.7%) from the omental cell screens showed an increase in lipolysis and were selected to determine if their primary activity could be confirmed. Human adipocytes were treated with 20 and 50 μg/ml of the botanical extracts for 4 h and the amount of FFA released was determined. Additionally, the extracts were tested to determine if they interfered with the assay reagents to generate a false-positive signal by incubating them with the reagents alone. Only 4 of the 23 extracts selected for additional testing had their initial activity confirmed and were not observed to significantly interfere with the assay reagents or induce cytotoxicity (data not shown). The four identified extracts were KPL_00107E1, KPL_00248E12, KPL_00120E1, and UPL_00042E9.

The four extracts were serially diluted to determine their dose-response effect on adipocyte lipolysis in both subcutaneous and omental cells. Extract KPL_00120E1 displayed the largest increase in lipolysis and lowest EC\textsubscript{50} in both subcutaneous and omental adipocytes (Table 1). The remaining extracts displayed dose-dependent increases in lipolysis in both cell types compared to the vehicle control. Only KPL_00120E1 and KPL_00248E12 displayed EC\textsubscript{50} values below 15 μg/ml and robust lipolysis without cytotoxicity. Three extracts were identified from the primary subcutaneous cell screen to inhibit basal lipolysis without inducing cytotoxicity; however, these extracts did not confirm their activity in follow-up assays.

### 3.4. Identification of botanical extracts with lipogenic properties using human primary subcutaneous preadipocytes

The same 580 botanical extracts were tested for their effects on human subcutaneous adipocyte lipogenesis using partially differentiated adipocytes. Extracts were added to 1-week-old partially differentiated ASCs and incubated with cells for 7 days. This second 7-day period is when differentiated adipocytes accumulate large amounts of triglyceride through lipogenesis. Extract concentrations of 50 μg/ml were used to maximize the response while limiting the cytotoxic effect of DMSO (0.25%) [34]. 10 ng/ml TNFα served as a positive control, inhibiting lipogenesis and triglyceride accumulation by 65% without cytotoxicity [34]. Lipogenesis and cytotoxicity were determined for each extract in separate cell-based screens. Fig. 4 shows the combined results of the two screens displaying triglyceride levels for both cytotoxic and noncytotoxic extracts. The lipogenesis screen gave a Z\textsuperscript{-} = 0.61. Forty-four extracts (7.6%) actively inhibited lipogenesis without inducing cytotoxicity and two induced lipogenesis. Fourteen of the initial actives from the primary screen were confirmed in a secondary inhibition assay and were retested in a dose response in subcutaneous and omental cells. Maximal inhibition of lipogenesis and induced cytotoxicity effects (and their IC\textsubscript{50} concentrations) are shown for eight extracts in Table 2. Three extracts (KPL_00752E12, KPL_00752E7, and KPL_00752E4) displayed dose-response inhibition of lipogenesis that was clearly separated from cytotoxicity effects. The two extracts that enhanced lipogenesis appear to contain FFAs and may have increased lipogenesis by increasing exogenous lipid concentration in the treatment medium.

### 4. Discussion

High throughput screening is the mainstay of drug discovery often relying on well-defined targets using miniaturized in vitro assays to allow rapid testing of hundreds of thousands of compounds. Cell-based assay activities also provide a robust screening system presenting the drug target in a more relevant context [35,36]. These cell-based assay systems are typically constructed to enhance screening parameters while sacrificing the normal biological context of the target. One way to overcome this drawback is to use primary cells containing the target of interest. With recent advancements in human primary cell isolation and propagation, large numbers of high-quality cells are readily available for drug discovery [37].

The current study demonstrates the potential of primary human ASC cultures to be used in primary compound or botanical screens. Specifically, we demonstrate that both undifferentiated and differentiated adipocyte cultures of primary human ASCs derived from subcutaneous and visceral/omental adipose depots can be used to efficiently screen botanical extracts in vitro. This indicates that the human primary ASC cultures can be adapted to a 384-well plate format and used as a robust alternative to murine preadipocyte cell lines such as 3T3-L1. Additionally, the use of a mixed donor lot can generate the billions of cells required for large screens and greatly

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**Table 1**

<table>
<thead>
<tr>
<th>Extract ID</th>
<th>Subcutaneous (EC\textsubscript{50})</th>
<th>Omental (EC\textsubscript{50})</th>
<th>Cytotoxicity (50 μg/ml)</th>
<th>Species</th>
<th>Plant part</th>
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<td>Max</td>
<td>Max</td>
<td>EC\textsubscript{50}</td>
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<tr>
<td>KPL_00120E1</td>
<td>43%</td>
<td>47%</td>
<td>9.8</td>
<td>12%</td>
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<td>28%</td>
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<td>18%</td>
<td>Alcea nudiflora (Lindl.) Boiss.</td>
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<tr>
<td>KPL_00248E12</td>
<td>32%</td>
<td>41%</td>
<td>13</td>
<td>10%</td>
<td>Cirsium ochrolepidium Juz.</td>
</tr>
<tr>
<td>UPL_00042E9</td>
<td>29%</td>
<td>48%</td>
<td>&gt;100</td>
<td>15%</td>
<td>Allium inopscipicum Vved.</td>
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<tr>
<td>Isoproterenol</td>
<td>100%</td>
<td>100%</td>
<td>30 nM</td>
<td>8%</td>
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</tbody>
</table>

EC\textsubscript{50} values are expressed as micrograms per milliliter. Max is maximum percent effect. Iso Max was 64.8 and 32.9 mM for subcutaneous and omental, respectively.

KPL denotes Kyrgyzstan plants; UPL denotes Uzbekistan plants.
reduce cell costs while averaging out the donor-to-donor variability inherent in human primary cells. While botanical extracts provide an available source of novel bioactive molecules, the use of unfraccionated mixtures complicates primary screening [38]. We have taken a systems approach by using human primary cell-based assays to identify active extracts. This limits the bias of preselecting the target; however, it adds complications of its own including possible cytotoxicity, cell impermeability and metabolic activation of the extracts. Successful identification of active crude extracts requires a robust assay and can test the limits of a primary screen. Our results suggest human primary ASCs provide a relevant and robust primary cell-based screening system. The primary and secondary screens of over 500 botanical extracts identified unique subsets with prolipolytic and antilipogenic activities that may eventually be useful in treating metabolic disorders.

The plant specimen collection used in this study contains a comprehensive sampling of Central Asia’s (Kazakhstan, Kyrgyzstan, Tajikistan and Uzbekistan) plant biodiversity. Emphasis was placed on endemic plants and plants that either belong to families known for high natural product production or are known as local medicinal plants. In addition, plants of the same species were collected from various extreme environmental conditions under the premise that plants exposed to different stresses often contain different inducible natural products with different bioactivities. All collected samples are vouchered as herbarium specimens.

Two extracts with relatively potent lipolytic effects were identified in our screen and are derived from Aconitum soongaricum, a relative of wolfsbane, and C. ochrolepidium, a relative of the common garden thistle. This Aconitum species is known to contain potent alkaloids with bioactive properties including immunological and antiarrhythmia activities [39–43]. Recently, a root extract from another Aconitum species, A. carmichaeli Debeaux (Ranunculaceae), was found to have profound effects on the metabolic function of brown adipose tissue (BAT) in cold-stressed mice [44]. The authors demonstrated that the extract enhanced UCP-1 gene expression and maintained the animal’s core body temperature by increasing brown adipose tissue at the expense of white adipose. A similar induction of lipolysis to liberate fatty acids for fuel consumption may be occurring in the adipocytes treated with the A. soongaricum extract (KPL_00120E1). Likewise, the Circium ochrolepidium Juz. extract increased lipolysis in both subcutaneous and omental adipocytes (KPL_00248E1); however, extracts of this particular species have not been studied extensively. A different Circium species, C. oligophyllum, has been shown to contain bioactive components that activate lipolysis in cultured rat adipocytes [45]. Additionally, oral administration of the extract reduced fat mass and overall body weight in the rats, increasing UCP-1 gene expression both in white adipose and brown adipose tissue. It is highly likely that a similar effect was identified in our screen from the Circium extract that we tested. The identification of these extracts from genera with known adipocyte activities further validates the utility of this screening system.

Three extracts from Acer negundo L. were found to have potent antilipogenic properties which did not represent aspects of cytotoxicity. These extracts were derived from different parts of the plant, with the leaf and above-ground extracts showing more potency than the stem and twig extract. This species of maple has been found to contain flavonoids and other potentially bioactive compounds [46–50]; however, extracts from A. negundo have not been studied for their effects on obesity. Another species of maple, A. tegmentosum, has been used in Korean traditional medicine to treat hepatic disorders including cirrhosis, hepatitis and cancer [51,52]. Some of these effects are attributed to the antiinflammatory and antioxidant activities of some of the flavonoids found in the extracts. It seems likely that the A. negundo extracts may possess similar flavonoids that may be responsible for its ability to reduce lipogenesis. Several flavonoids have been found to inhibit lipogenesis in adipocytes and other cells through a variety of mechanisms including inhibiting fatty acid synthase or nuclear receptors [53–55]. While we have not identified the active components of these extracts, there is reason to suspect similar bioactive components already known to inhibit adipocyte lipogenesis.

Subcutaneous and visceral adipose depots differ with respect to their cellular composition and expression of adiponectins and

<table>
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<th>Extract ID</th>
<th>Inhibition</th>
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proinflammatory growth factors [24,32]. Their relative abundance is believed to play a contributory role to the pathogenesis of insulin resistance and the metabolic syndrome. Increased visceral adiposity, as compared to subcutaneous adiposity, has been correlated with the onset and severity of hyperglycemia, hypertriglyceridemia and related comorbidities of the metabolic syndrome. For these reasons, focusing on the use of visceral/omenta-derived rather than on subcutaneous-derived ASCs may have particular advantages as far as determining more precise pathophysiologic roles and may be more amenable for further development of drug discovery and botanical extract screening. In the current study, visceral ASCs were used in a limited primary screen and as a secondary counterscreen; however, they can be easily expanded and formatted for use in much larger primary screens. While their lipolytic responses were very similar, the lipogenic responses between omental and visceral adipocytes, mouse adipose explants, and normal mice. Endocrinology 2005;146:2209–31.

Botanical extracts offer advantages and challenges both as adjunctive therapies and as a source of future pharmaceuticals. Plants serve as a rich source of diverse chemical compounds, that is, bioactives, which, theoretically, can be extracted with appropriate methods, thereby reducing the cost of both discovery and manufacture. Coupling renewable botanical extracts with human primary cell screening technology represents a rapid and economical method to identify natural products that can become therapeutics.

References


