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A polyphenolic extract from green tea leaves activates fat browning in high-fat-diet-induced obese mice

Audrey M. Neyrinck[a], Laure B. Bindels[a], Lucie Geurts[a,b], Matthias Van Hul[a,b], Patrice D. Cani[a,b], Nathalie M. Delzenne[a,*]

[a]Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, Brussels, Belgium
[b]Walloon Excellence in Life sciences and BIOtechnology (WELBIO), Louvain Drug Research Institute, UCL, B-1200 Brussels, Belgium

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Abstract

Fat browning has emerged as an attractive target for the treatment of obesity and related metabolic disorders. Its activation leads to increased energy expenditure and reduced adiposity, thus contributing to a better energy homeostasis. Green tea extracts (GTEs) were shown to attenuate obesity and low-grade inflammation and to induce the lipolytic pathway in the white adipose tissue (WAT) of mice fed a high-fat diet. The aim of the present study was to determine whether the antiobesity effect of an extract from green tea leaves was associated with the activation of browning in the WAT and/or the inhibition of whitening in the brown adipose tissue (BAT) in HF-diet induced obese mice. Mice were fed a control diet or an HF diet supplemented with or without 0.5% polyphenolic GTE for 8 weeks. GTE supplementation significantly reduced HF-induced adiposity (WAT and BAT) and HF-induced inflammation in WAT. Histological analysis revealed that GTE reduced the adipocyte size in the WAT and the lipid droplet size in the BAT. Markers of browning were induced in the WAT upon GTE treatment, whereas markers of HF-induced whitening were reduced in the BAT. These results suggest that browning activation in the WAT and whitening reduction in the BAT by the GTE could participate to the improvement of metabolic and inflammatory disorders mediated by GTE upon HF diet. Our study emphasizes the importance of using GTE as a nutritional tool to activate browning and to decrease fat storage in all adipose tissues, which attenuate obesity.

Keywords: Browning; Brown adipose tissue; Whitening; Green tea; Obesity; High-fat diet

1. Introduction

Obesity plays a central role in the development of diabetes and is known to increase the risk of death, predominantly from cardiovascular disease. Many studies have shown that chronic low-grade adipose inflammation, resulting from an imbalance of adipokine production from the white adipose tissue (WAT), contributes to systemic metabolic dysfunction [1]. In contrast to the WAT, the brown adipose tissue (BAT) is a highly vascularized organ that is rich in mitochondria [2]. BAT mitochondria are enriched in uncoupling protein-1 (UCP-1) and produce heat by uncoupling the respiratory chain [3,4]. The BAT protects against hypothermia in small mammals and newborn infants through thermogenesis [5]. Studies using positron emission tomography demonstrated that metabolically active brown fat is also present in adults [3,6–9]. Not surprisingly, BAT thermogenesis also contributes to whole-body energy balance. Thus, genetic ablation of brown fat fosters the development of obesity, whereas activation of the BAT thermogenesis has been linked to increased energy expenditure, reduced adiposity and lower plasma lipids [3,4]. The recognition that functional brown fat is present in adult humans raises the prospect that brown fat abundance and/or function may be augmented to improve energy balance in obesity and to treat obesity-associated metabolic disorders. Several studies have analyzed the browning process of the WAT as a means to tackle obesity, but only a few studies have focused on the impact of molecular mechanisms that contribute to obesity-linked BAT dysfunction. This process is associated with the whitening of the BAT, that is, the accumulation of large lipid droplets in brown fat cells and a decrease of mitochondria number and mitochondrial dysfunction [10,11]. Indeed, BAT function declines with obesity, giving it a whitened appearance, but the mechanisms that contribute to this decline have been incompletely defined [10,11].

The life cycle of an adipocyte begins at the stage of a multipotent stem cell, which can differentiate into multiple cell types, including myoblasts and adipocytes. Intriguingly, unlike the majority of white adipocytes, the adipocytes found in BAT share a common precursor with skeletal muscle [12]. Expression of various transcriptional regulators such as peroxisome proliferator-activated receptor gamma (PPARγ) drives the differentiation of adipocytes. PPARγ is a hormone receptor specific to adipocytes that has been implicated as a key enhancer of adipogenesis. CCAAT enhancer binding protein

* Corresponding author at: Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, 73 av. E. Mounier B1.73.11, B-1200 Brussels, Belgium. Tel.: +32 2 764 7369.
E-mail address: nathalie.delzenne@uclouvain.be (N.M. Delzenne).

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(C/EBPα) α and PPARγ are the key transcription factors that work together to in turn activate a group of genes that promote adipogenesis (reviewed in [13]). Interestingly, PPARγ not only is involved in white adipogenesis but also plays a key role in the induction of brown adipocyte-specific genes (reviewed in [14]). PR domain containing 16 (PRDM16) is also a determining transcription factor for BAT development by partnering with peroxisome proliferator-activated receptor γ coactivator (PGC-1) α, which is a coactivator of PPARγ, and it primarily controls mitochondrial biogenesis through the induction of uncoupling proteins such as UCP-1 [12].

The use of natural products for medical purposes dates back thousands of years; however, their use in the discovery and development of modern drugs has only occurred since the early 19th century [15,16]. Natural products have been studied for their role in the regulation of adipocyte life cycle. Recently, a wealth of phytochemicals showing fat-browning activity has been reported (reviewed in [15,17]). Phytochemicals can target different stages in the adipocyte life cycle by decreasing adipogenesis, by inducing lipolysis, by inducing adipocyte apoptosis and by inducing transdifferentiation of white to brown-like adipocytes [15,17,18].

Camellia-based teas are one of the most widely consumed beverages in the world. Their popularity is in part due to the fact that they contain a substantial amount of the purine alkaloid caffeine and trace amounts of theobromine [19]. In 2010, ~3.2 million metric tons of dried tea were produced, 61% of which was black tea and 31% green tea. In all cases, the raw material is young leaves, the tea flush. Epidemiological evidence and experimental studies suggest that drinking green tea is associated with a lower risk of obesity and related diseases [20,21]. Green tea is made from the dried leaves of the *Camellia sinensis* plant. Different from fermented black tea and partially fermented oolong tea, green tea is a nonfermented tea that is produced from direct drying of fresh green tea leaves by hot steam and air [22]. During this process, polyphenol oxidase is inactivated and polyphenols are preserved [22]. Compared to black tea and oolong tea, green tea contains the highest amount of phytochemicals such as catechins, the major polyphenols in green tea that constitute about 10% to 35% of its total dry weight [17,22]. The most abundant green tea catechins are epigallocatechin gallate (EGCG), which accounts for about 50% to 70% of green tea catechins [17,22]. Several studies have previously reported that green tea (*C. sinensis*, Theaceae) and its major polyphenol EGCG have obesity preventive effects (reviewed in [20,22]). Green tea, green tea catechins, and EGCG have been demonstrated in cell culture and animal models of obesity to reduce adipocyte differentiation and proliferation, lipogenesis, fat mass, body weight, fat absorption, plasma levels of triglycerides, free fatty acids, cholesterol, glucose, insulin and leptin (reviewed in [17]). Different mechanisms have been proposed to explain the antiobesity effects of green tea or its polyphenolic compounds including modulation of pancreatic lipase, inhibition of *de novo* lipogenesis, and enhanced fatty acid oxidation and BAT thermogenesis [21–23]. Our study shows for the first time positive effects of green tea extract (GTE) on genes related to the conversion of WAT to BAT and the inhibition of whitening in the BAT in obese mice fed a high-fat (HF) diet.

2. Material and methods

2.1. Animals and diet intervention

Male C57BL/6J mice (9 weeks old) were purchased from Janvier Labs (France) and maintained in specific pathogen-free environment. Animals were housed in groups of three mice per cage in a controlled environment (12-h daylight cycle) with free access to food and water. Animal experiments were approved and performed in accordance with the guidelines of the local ethics committee. Housing conditions were as specified by the Belgian Law of 29 May 2013 on the protection of laboratory animals (Agreement LA 1230314). The agreement of the animal experiments performed in this study was given by the local Ethical Committee under the specific number 2010/UCL/MD/022.

After 1 week of acclimatization, mice were randomly assigned into three groups: a group fed a control diet (D12450K, 10% fat, 70% carbohydrates; Research Diet, New Brunswick, NJ, USA), a group fed an HF diet (60% fat, D12492, Research Diet) and a group fed an HF diet supplemented with 0.5% green tea leaves extract. Extract of green tea leaves was supplied by Oxylent (Ghislenghien, Belgium) and was composed at 95% of polyphenols (Folin–Ciocalteu method, equivalent gallic acid), with the concentrations of catechins and EGCG being 60% and 30%, respectively (high-performance liquid chromatography detection). Body weight, food intake and water consumption were recorded twice a week. Body composition was assessed every 2 weeks using 7.5-MHz time-domain NMR (TD-NMR) (LF50 Minispec, Bruker, Rheinstetten, Germany). After 8 weeks of dietary treatment, 6-h-fasted mice were anesthetized with isoflurane gas (Abbot, Ottignies, Belgium). Blood samples were harvested for further analysis. Mice were necropsied after cervical dislocation. Adipose tissues (epididymal, subcutaneous, visceral, interscapular) were weighted, collected, frozen in liquid nitrogen and stored at −80°C. Pieces of subcutaneous WAT (sWAT) and interscapular BAT (iBAT) were fixed in 4% formaldehyde for further histological analyses.

2.2. Tissue mRNA analyses

Total RNA was isolated from tissues using the TriPure isolation reagent kit (Roche Diagnostics, Penzberg, Germany). For adipose tissue, RNA quality was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) with a quality threshold at 6. Complementary DNA was prepared by reverse transcription of 1 μg total RNA using the Kit Reverse Transcription System (Promega, Madison, WI, USA). Real-time polymerase chain reaction (PCR) was performed with a StepOnePlus Real-Time PCR System and software (Applied Biosystems, Den Ijssel, the Netherlands) using SYBR Green (Applied Biosystems and Eurogentec, Verviers, Belgium) for detection. All samples were run in duplicate in a single 96-well reaction plate, and data were analyzed according to the $2-\Delta\Delta CT$ method. The purity of the amplified product was verified by analyzing the melting curve performed at the end of the amplification step. The ribosomal protein L19 (*Rpl19*) gene was chosen as a reference gene. The primer sequences of the targeted genes are given in Supplemental Table S1.

2.3. Histological analysis

The tissues were fixed in 4% formaldehyde. Hematoxylin and eosin staining was performed using standard protocols on 5-μm adipose tissue sections. Adipocyte size in the sWAT and lipid droplets in the iBAT were estimated in a blind manner, and a score was assigned. UCP-1 staining (ab23841, Abcam) was determined as previously described [24,25]. Whole-mount sections were digitized using a whole-slide scanner (Leica SCN400, Leica Microsystems, Germany), and images were captured using the Leica Image Viewer Software (Version 4.0.4). The UCP-1-positive area (% *versus* total area) was determined in a blind manner by quantifying the amount of stained area using ImageJ software (version 1.48r; National Institutes of Health, Bethesda, MD, USA). Five slices per mouse were counted for all histological analysis [24].

2.4. Statistical analysis

Results are presented as means with their standard errors. Statistical analysis was performed by one-way or two-way analysis of variance (ANOVA) followed by *post hoc* Tukey or Bonferroni multiple comparison tests, respectively, using the GraphPad Prism software (version 5.00; GraphPad Software, San Diego, CA, USA); unpaired Student's *t* test was used to compare HF+GTE group *versus* HF group, when indicated. The results were considered statistically significant when P value was $<.05$.

3. Results

3.1. GTE supplementation reduces HF-induced obesity and adiposity

HF diet feeding significantly increased the adiposity, the body weight gain and the caloric intake compared to the control diet without affecting the lean mass evolution (Fig. 1). Supplementation with GTE reduced obesity and fat mass expansion, reaching significant P value ($P<.05$) after 38 days of treatment (Fig. 1A and C). The total caloric intake was not modified by the GTE supplementation (Fig. 1D), thereby suggesting a change in energy metabolism rather than changes in appetite as an explanation of the effect of GTE on body weight.

Fat mass development due to the HF diet was confirmed by an increased weight of WAT (visceral, subcutaneous and epididymal) and the iBAT *versus* the control group (Fig. 2). GTE treatment significantly reduced epididymal and subcutaneous adipose tissues weight (Fig. 2B and C), and GTE completely normalized the iBAT weight (Fig. 2D). To better explain the decreased fat mass observed following the GTE treatment, we performed histological analyses after hematoxylin–