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Adipose tissue diacylglycerol acyltransferase activity and blood lipoprotein triglyceride enrichment in women with abdominal obesity

Julie Anne Côté [a,b], Mélanie Nadeau [a,b], Mathieu Leboeuf [c], Line Blackburn [c], André Tchernof [a,b,*]

[a] Endocrinology and Nephrology, Laval University Medical Research Center, Quebec, Canada
[b] Department of Nutrition, Laval University, Quebec, Canada
[c] Gynecology Unit, Laval University Medical Research Center, Quebec, Canada

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ABSTRACT

Previous studies have suggested altered triglyceride (TG) storage in patients with abdominal obesity and blood lipid disorders.
Objective: We hypothesized that women with abdominal obesity and a dysmetabolic profile have low DGAT activity in their abdominal fat compartments.
Methods: Paired omental (OM) and subcutaneous (SC) adipose tissue samples were obtained surgically from 39 women undergoing abdominal hysterectomies. Body composition and fat distribution were measured by dual energy x-ray absorptiometry and computed tomography. DGAT activity was measured by acylation of sn-l,2-diacylglycerol with [^{14}C] oleoyl-CoA in microsomal fractions isolated from whole adipose tissue homogenates. DGAT activity was calculated on the basis of picomoles (pmol) TG synthesized in the assay per min per mg lipid, per μg protein or per 1000 cells.
Results: No depot differences were found when DGAT activity was reported per μg microsomal protein or per 1000 cells. DGAT activity in either depot was not associated with adipocyte diameters and blood lipid profile variables. DGAT activity per mg lipid was higher in OM than in abdominal SC adipose tissue (0.43 ± 0.20 *vs.* 0.34 ± 0.18 pmol/min/mg lipid, $p < 0.05$). OM DGAT activity was negatively correlated with OM adipocyte diameter and visceral adipose tissue area ($r = -0.43$, $p < 0.01$ and $r = -0.38$, $p < 0.05$ respectively). Plasma total, LDL and HDL TG levels were negatively associated with OM DGAT activity independent of total body fat mass ($r = -0.39$, $p < 0.05$, $r = -0.46$, $p < 0.001$ and $r = -0.40$, $p < 0.05$ respectively).
Conclusion: A defect in adipose tissue DGAT activity is predictive of adiposity and blood lipoprotein TG enrichment only when considering activity per tissue lipid mass.

1. Introduction

Triacylglycerols or triglycerides (TGs) accumulate in cytosolic lipid droplets of the adipocyte. In humans and other species, two major pathways have been elucidated for endogenous TG synthesis: 1) the glycerol phosphate pathway; and 2) the monoacylglycerol pathway. While the former is found in most cells, the latter takes place in specific cell types such as the enterocyte, hepatocyte and adipocyte. The final reaction of both pathways is the acylation of a sn-1,2-diacylglycerol at the sn-3 position with a fatty acyl-CoA as substrate [1]. This reaction is catalyzed by acyl-CoA: diacylglycerol acyltransferase enzymes [2]. The DGAT1 and DGAT2 genes were identified and were found to be integral membrane proteins of the endoplasmic reticulum [2,3].

TGs are physiologically essential but excessive accumulation of these lipids in adipose and non adipose tissues leads to the development of obesity and obesity-related complications such as diabetes, cardiovascular diseases and dyslipidemia [4]. For any given amount of total body fat mass, individuals with excess adipose tissue in visceral fat compartments are at higher risk of suffering from metabolic diseases [5]. However, the mechanisms by which some depots expand to a greater extent than others have not been fully elucidated. Considering that DGAT enzymes catalyze the final step in TG biosynthesis and that visceral obesity is often associated with excess postprandial TG excursions and impaired TG storage [6,7], depot differences and obesity-related variation in

* Corresponding author. Molecular Endocrinology and Oncology Research Center, Laval University Medical Research Center, 2705 Laurier Blvd. (T3-67), Québec, Qc, Canada G1V 4G2. Tel.: +1 418 654 2296; fax: +1 418 654 2761.
E-mail address: andre.tchernof@crchul.ulaval.ca (A. Tchernof).

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DGAT activity could influence fat storage and contribute to the dysmetabolic state of visceral obesity.

Few studies have assessed DGAT activity in humans. Hou et al. demonstrated that DGAT activity was higher in visceral compared to SC fat in non-obese patients, while no depot differences were found in obese subjects. DGAT activity expressed per mg of tissue lipid was negatively correlated with OM adipocyte size and positively associated with fatty acid storage rates [8]. To the best of our knowledge, no study has ever examined the association between DGAT activity and the metabolic profile. Thus, our objective was to examine the relationship between DGAT activity and total adiposity, as well as body fat distribution, adipocyte size and the metabolic profile, with a particular emphasis on blood lipoprotein composition. We hypothesized that women with abdominal obesity and a dysmetabolic profile have low DGAT activity in either abdominal fat compartment.

2. Subjects and methods

2.1. Subjects

Women were recruited through the elective surgery schedule of the Gynecology Unit at Laval University Medical Research Center. Women ($n = 39$) electing for abdominal gynecological surgery aged 40–60 years were included in the present analysis. Participants were scheduled for total ($n = 38$) or subtotal ($n = 1$) abdominal hysterectomies, or myomectomy ($n = 1$), sometimes accompanied by salpingo-oophorectomy of one ($n = 7$) or two ($n = 8$) ovaries. Reasons for surgery included one or more of the following: menorrhagia ($n = 5$), endometriosis ($n = 3$), uterine fibroids ($n = 7$), ovarian cyst ($n = 4$), incapacitating dysmenorrhea ($n = 6$), pelvic pain ($n = 2$), endometrial hyperplasia ($n = 2$), endometrial polyp ($n = 1$), vaginal polyp ($n = 1$), pelvic adhesions ($n = 2$), adenomyosis ($n = 1$), anemic menorrhagia ($n = 9$), sigmoid adherence ($n = 1$), uterine leiomyoma ($n = 8$), uterine myoma ($n = 18$), mucinous cystadenoma ($n = 1$), abdominal pain ($n = 1$), left hydrosalpinx ($n = 1$), right adnexal mass or uterine myomatosis ($n = 1$). This project was approved by the medical ethics committee of Laval University Medical Research Center. Before their inclusion in the study, all women provided written informed consent.

2.2. Body fatness and body fat distribution measurements

These tests were performed a few days before surgery. Total body fat mass, body fat percentage and lean body mass were determined by dual energy x-ray absorptiometry (DEXA). Computed tomography (CT) was used to measure abdominal subcutaneous and visceral adipose tissue areas (SAT and VAT respectively) [9] using a GE Light Speed 1.1 CT scanner (General Electric Medical Systems, Milwaukee, WI). Subjects were examined in the supine position with arms stretched above the head. The scanning position was established at the L4-L5 vertebrae level with a scout image of the body. VAT area was quantified by delineating the intra-abdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls and the posterior aspect of the vertebral body using Image J 1.33u software (National Institutes of Health, USA). Adipose tissue was identified using an attenuation range of −190 to −30 Hounsfield Units. Inter-observer coefficients of variation were 0.33% and 1.33% for SAT and VAT areas respectively ($n = 10$).

2.3. Adipose tissue sampling

Paired OM and SC adipose tissue samples were obtained during surgery and immediately carried to the laboratory. Part of the sample was used for adipocyte isolation and the remaining tissues were frozen at −80 °C for further analyses.

2.4. Adipocyte isolation and cell size measurement

Adipose tissue samples were digested with collagenase type I in Krebs-Ringer-Henseleit (KRH) buffer for up to 45 min at 37 °C according to a modified version of the Rodbell method [10]. Adipocyte suspensions were filtered through nylon mesh and washed 3 times with KRH buffer. Pictures of adipocyte suspensions were taken using a phase contrast microscope connected to a camera and computer interface. The Scion Image software (Scion Corporation, Frederick, MA) was used to measure the diameter of 250 adipocytes in each fat depot.

2.5. Lipid profile and glucose homeostasis

Blood samples were obtained after a 12-h fast on the morning of surgery. Cholesterol and TG level measurements in plasma and in lipoprotein fractions were performed with the Olympus AU400 (Beckman Coulter, Mississauga, Canada). Plasma VLDL were isolated by ultracentrifugation [11] and the HDL fraction was obtained after precipitation of LDL in the infranatant with heparin and $MnCl_2$ [12]. Cholesterol content of the infranatant was measured before and after precipitation and LDL cholesterol concentration was obtained by difference. Apo B and apo A1 were measured using the Siemens Healthcare Diagnostics BN ProSpec (Siemens Healthcare Diagnostics, Mississauga, Ontario, Canada). Fasting insulin and blood glucose were measured in presurgery blood samples. Glucose was measured with a Modular P800 system (Roche, Diagnostics, Laval, Canada). Insulin was measured by ELISA (Alpco, Salem, NH, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L) ÷ 22.5 [13].

2.6. Preparation of the microsomal fraction

Approximately 100–120 mg whole OM and SC adipose tissue samples were weighted and put on dry ice. One mL of homogenization buffer (TAE buffer) with 255 mM sucrose, 20 mM Tris-HCl, pH 7.5, 1 mM EDTA and anti-protease tablets from Roche (Indianapolis, IN) was added to each sample. Samples were vortexed twice for 10 s and TAE buffer was removed to eliminate excess blood. One mL of TAE buffer was added and samples were homogenized by 5 up-and-down strokes at medium speed with a Potter-Elvehjem. The homogenates were centrifuged 10 min at 3100 rpm (4 °C). The supernatants were further centrifuged for 20 min at 15600 rpm while the fat cake was saved and left in the tube for weighting and control of the assay. Any remaining lipids were collected again and the cytosolic fraction was centrifuged at 49 600 rpm for 45 min to collect the microsomal fraction. The pellet from this centrifugation was resuspended in 60 μL TAE buffer. An aliquot of 5 μL was used to measure protein content with the BCA protein assay (Pierce; Rockford, IL, USA). The fat cakes collected during the isolation process were pooled and extracted with the Dole procedure. Lipids extracted were weighed and used as the amount of total tissue lipids in the assay.

2.7. Measurement of DGAT activity

We used a modification of the assay by Coleman [14]. The assays were performed in 16 × 100 mm glass tubes. Activity was assessed in duplicates. Each individual reaction mixture contained the following, in a total volume of 80 μL: 0.12 M of the Tris, 8 mM $MgCl_2$, 1 mg/L albumin, 4 μg microsomal protein, 0.2 mM diolein (Sigma–