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Oxidative activity of 17β-hydroxysteroid dehydrogenase on testosterone in male abdominal adipose tissues and cellular localization of 17β-HSD type 2

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ABSTRACT

Testosterone can be converted into androstenedione (4-dione) by 17β-hydroxysteroid dehydrogenase (HSD) activity likely performed by 17β-HSD type 2. Our objective was to evaluate the rate of testosterone conversion to 4-dione as well as expression and localization of 17β-HSD type 2 in omental (OM) vs. subcutaneous (SC) adipose tissues of men. Formation of 4-dione from testosterone was significantly higher in homogenates ($p \leq 0.001$) and explants ($p \leq 0.01$) of OM than SC tissue. Microscopy analyses and biochemical assays in cell fractions localized the enzyme in the vasculature/endothelial cells of adipose tissues. Conversion of testosterone to 4-dione was weakly detected in most OM and/or SC preadipocyte cultures. Positive correlations were found between 17β-HSD type 2 activity in whole tissue and BMI or SC adipocyte diameter. We conclude that conversion of testosterone to 4-dione detected in abdominal adipose tissue is caused by 17β-HSD type 2 which is localized in the vasculature of the adipose compartment.

1. Introduction

Androgens likely modulate body fat distribution in men and women [reviewed in (Blouin et al., 2009a, 2009b; Zerradi et al., 2014)]. Intra-abdominal fat accumulation is negatively correlated with circulating androgens in both sexes (Blouin et al., 2005; Cote et al., 2012) and testosterone replacement in men leads to lower waist circumference and improved metabolic profile (Haider et al., 2014). From the cellular standpoint, androgens inhibit preadipocyte differentiation (Blouin et al., 2008; Chazenbalk et al., 2013; Gupta et al., 2008). Testosterone also has a negative effect on mitochondrial biogenesis through promoting a decrease in mitochondrial mass in 3T3-L1 adipocytes (Capllonch-Amer et al., 2014). Lipolysis is increased in cultured adipocytes after androgen treatment [Reviewed in (Blouin et al., 2008)]. Testosterone also induces a decrease in adiponectin receptor and adiponectin expression which is consistent with an inhibitory effect on lipid accumulation and fat cell differentiation (Capllonch-Amer et al., 2014).

17β-hydroxysteroid dehydrogenases (17β-HSDs) play an important role in the biological activity of steroid hormones such as estrogens and androgens by catalyzing the reduction of 17-ketosteroids or the oxidation of 17β-hydroxysteroids with NAD(P)H or $NAD(P)^+$ as cofactor, respectively (Luu-The, 2001). Enzyme activities associated with the various 17β-HSD isoforms are widespread in human tissues, not only in classic steroidogenic tissues such as the testis, ovary, and placenta, but also in a large number of peripheral sites including adipose tissue [reviewed in (Lin et al., 2013; Tchernof et al., 2014)].

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Among 17β-HSD isoenzymes, 17β-HSD type 2 is a transmembrane protein which has an unknown 3D-structure. It catalyzes the conversion of active 17β-hydroxysteroids into less active 17-ketosteroids with NAD + as cofactor, which decreases tissue levels of active estrogens and androgens (Wetzel et al., 2011). Specifically, it is responsible for the conversion of testosterone to androstenedione (4-dione), of estradiol (E_2) to estrone (E_1) and of 20α-dihydroprogesterone to progesterone (Wu et al., 1993). The enzyme also exhibits 3β-HSD activity in intact cells and homogenates of transfected HEK 293 cells (Suzuki et al., 2000). 17β-HSD type 2 is expressed in liver, placenta, endometrium and small intestine. It may be involved in maintaining the level of progesterone in pregnancy by inactivating androgens and estrogens in the placenta (Wu et al., 1993). The enzyme also likely serves as a barrier that lowers E_2 secretion rates toward the foetal blood circulation (Drolet et al., 2007).

Little data are available on 17β-HSD type 2 in human abdominal adipose tissues. We have initially shown that 17β-HSD type 2 mRNA is more strongly expressed in omental (OM) adipose tissue than in subcutaneous (SC) adipose tissue of women (Blouin et al., 2009a, 2009b). Our objective was to evaluate the rate of testosterone conversion to 4-dione as well as expression and localization of 17β-HSD type 2 in human OM vs. SC adipose tissues obtained from male subjects at the time of surgery. We hypothesized that the conversion of testosterone to 4-dione is present in OM and SC adipose tissues in men and that 17β-HSD type 2 is responsible for this activity.

2. Materials and methods

2.1. Subjects

The study sample included obese and non-obese men recruited through the elective general surgery schedule of the CHU de Québec Medical Center (Québec, Canada) and severely obese men undergoing bariatric surgery at the Quebec Cardiology and Pulmonology Institute (Québec, Canada). The project was approved by the ethics committees of both institutions. All subjects provided written informed consent.

2.2. Adipose tissue sampling, preadipocyte isolation and differentiation

During the surgical procedure, adipose tissue samples were collected at the site of the surgical incision (lower abdomen, SC adipose tissue) and at the greater omentum (OM adipose tissue) and immediately carried to the laboratory. A portion of the sample was used for tissue explants, another was frozen for gene expression or preparation of homogenates and finally, a portion was used for isolation of preadipocytes. Samples were digested for 45 min at 37 °C using type I collagenase in Krebs-Ringer-Henseleit buffer supplemented with 0.1 mg/ml ascorbic acid, 5 mM glucose, 0.1 μM adenosine, and 4% bovine serum albumin (BSA) according to a modified version of the Rodbell method (Rodbell, 1964). After tissue digestion, the cell suspension was filtered through nylon mesh and floating adipocytes were washed three times with Krebs-Ringer-Henseleit buffer. Preadipocytes were isolated from the stromal-vascular fraction using a modification of the Van Harmelen method (Van Harmelen et al., 2004). Briefly, the residual buffer, which contained the stromal-vascular fraction, was centrifuged and the pellet was washed in DMEM/F12. Preadipocytes were then filtered through a 140 μm nylon mesh and placed in culture plates at 37 °C under 5% CO_2 atmosphere. Cells were maintained in DMEM/F12 supplemented with 2.5% fetal bovine serum, 10 ng/ml epidermal growth factor, 1 ng/ml fibroblast growth factor, 6 μM insulin, 2.5 μg/ml amphotericin B and 50 μg/ml gentamicin. Medium was changed every 2–3 days. Differentiation of fully confluent preadipocyte cultures in 12-well plates was induced using commercial differentiation medium from Zen Bio (Durham, NC, USA), and followed for 14 days. Differentiation medium consisted of DMEM/F12 supplemented with a PPAR-γ agonist, insulin, dexamethasone and 3-isobutyl-1-methylxanthine.

2.3. Preparation of homogenates and cell/explant cultures

For homogenates, frozen adipose tissue samples (50 mg) were homogenized in sodium phosphate buffer, pH 7.4 (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 20% glycerol, 1 mM EDTA). The homogenates were centrifuged at 12,000 rpm to remove the lipid layer. 800 μl of sodium phosphate buffer supplemented with 1 mM β-NAD and 0.144 μM ^{14}C-testosterone (American Radiolabeled Chemicals, St. Louis, MO, USA) were added to 200 μl of tissue homogenates followed by 24 h incubation at 37 °C with shaking. For explants, fresh adipose tissue samples (100 mg) were cut into small pieces and incubated in M199 medium (30 mg tissue/ml) with ^{14}C-testosterone (0.1 μM) for 24 h at 37 °C under a 5% CO_2 atmosphere. OM and SC preadipocyte cultures were treated with ^{14}C-testosterone (0.03 μM and 0.1 μM) and incubated for 24 h at 37 °C under a 5% CO_2 atmosphere. For enzyme inhibition, preadipocyte cultures were treated with 10 μM 17β-HSD type 2 inhibitor EM-919 (Poirier et al., 2001). No toxicity of the inhibitor was detected on adipose tissue cultures using the Toxilight bioassay kit (Lonza, Allendale, NJ, USA). Fresh and frozen adipose tissue samples (130 mg) were homogenized in sodium phosphate buffer, pH 7.4 and centrifuged at 12,000 rpm to remove the lipid layer. 800 μl of sodium phosphate buffer supplemented with 2 mM β-NAD and 10 μM 17β-HSD type 2 inhibitor EM-919 were added to 200 μl of tissue homogenates followed by a 2 h pre-incubation at 37 °C with shaking before addition of ^{14}C-testosterone (0.144 μM or 0.216 μM) for 24 h of incubation.

2.4. Steroid extraction and TLC measurements

Steroids from tissue homogenates, explants and preadipocyte culture media were extracted twice with 2 ml diethyl ether followed by drying under nitrogen gas. Dichloromethane was used to dissolve the steroids which were applied to silica gel thin layer chromatography (TLC) plates. Plates were developed in a sealed glass chamber containing 100 ml of toluene/acetone (4:1) and scanned on a Storm 860 PhosphorImager (GE Healthcare, Baie D'Urfe, QC, Canada). The inactivation rate of testosterone was expressed as androstenedione (4-dione) formation in pmol/mg protein/24 h for homogenates and in pmol/mg explants/24 h for explants. Image J software was used for these calculations (NIH, Bethesda, MD, USA).

2.5. Measurement of mRNA expression by real-time RT-PCR

Total RNA was extracted from whole OM and SC tissue, and from non-differentiated and differentiated preadipocyte cultures using the RNeasy lipid tissues mini kit and on-column DNase treatment (Qiagen, Hilden, DE) following the manufacturer's instructions. Total RNA quality was assessed on an Agilent BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). First-strand cDNA synthesis was accomplished using Superscript III RNase H-RT (Invitrogen Life Technologies, Burlington, ON, Canada), oligo-dT_{18}, random hexamers and purified with PCR purification kit (Qiagen, Hilden, DE). cDNA corresponding to 20 ng of total RNA was used to perform fluorescent-based real-time RT-PCR quantification using the Light Cycler 480 (Roche Diagnostics, Mannheim, DE). The Light Cycler