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Chemerin gene expression is regulated by food restriction and food restriction–refeeding in rat adipose tissue but not in liver

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ABSTRACT

Chemerin is an adipokine that regulates adipocyte development and metabolism as well as inflammatory and immune function of some cells. Although chemerin may be linked to obesity and related diseases, little is known about the nutritional regulation of chemerin gene expression. We investigated the effect of prolonged food restriction, a common approach in treating obesity and related diseases, and prolonged food restriction–refeeding on chemerin gene expression in rat white adipose tissue and liver. The prolonged food restriction was accompanied by an approximately 2-fold decrease in chemerin mRNA level in rat white adipose tissue. Upon refeeding, an increase (approximately 8-fold as compared to rats maintained on restricted diet and 4-fold as compared to control) in chemerin mRNA level in white adipose tissue was found. Surprisingly, no effect of food restriction and food restriction–refeeding on chemerin mRNA level in the liver was found. Chemerin mRNA level in adipose tissue was positively correlated with serum insulin concentration. Moreover insulin increased significantly chemerin gene expression in primary rat adipocytes. The changes in chemerin mRNA level in adipose tissue and serum chemerin concentrations were associated with changes in serum leptin and free fatty acid concentrations. Collectively, the data presented here indicate that chemerin gene expression is regulated by nutritional status in rat adipose tissue but not in liver. It seems that insulin plays important role in stimulation of chemerin gene expression in adipose tissue. However, changes in serum leptin and free fatty acids concentrations after food restriction–refeeding suggest that the role of these factors in the regulation of chemerin gene expression in adipose tissue cannot be excluded. Lack of the effect of food restriction and food restriction–refeeding on liver chemerin gene expression suggests that adipose tissue is the dietary modifiable source of serum chemerin concentration.

1. Introduction

Chemerin, known as retinoic acid receptor responder 2 (RARRES2) or tazarotene-induced gene 2 (TIG2), has been initially identified as a product of gene that was up-regulated by the RARβ/γ-selective antipsoriatic synthetic retinoid tazarotene [1]. Chemerin is synthesized as an inactive protein (prochemerin), which undergoes proteolytic processing to biologically active form [2]. A number of reports suggest that chemerin is a natural ligand for G-coupled receptor chemokine like receptor (CMKLR, also known as chemR23 or GPCR-DEZ) [3,4]. More recent studies have found chemerin to be an adipokine induced during differentiation of 3T3-L1 cells [5–8]. It has been suggested that chemerin regulates both the adipocyte differentiation and the lipid metabolism in adipose tissue [7]. Moreover, it has been postulated that chemerin is involved in the regulation of lipogenesis [9], and can either activate insulin signaling in 3T3-L1 cells [8], or induce insulin resistance in human skeletal muscle cells [10]. In humans, circulating chemerin concentrations are closely associated with body mass index (BMI), serum triacylglycerol concentrations and blood pressure [5,6]. Recently published data indicates that serum chemerin concentration is positively correlated with C-reactive protein, which suggests that chemerin is associated with inflammation [11,12]. However, anti-inflammatory function of 15-oligomer C-terminal peptide derived from chemerin has been suggested by other studies [2,13].

Relatively little information is available concerning the factors affecting regulation of the gene expression in adipose tissue and some data are conflicting. Tan et al. [14] reported that in human adipose tissue explants chemerin production was significantly increased by insulin, decreased by metformin, and was not affected by the gonadal and adrenal steroids (testosterone, 17β-estradiol, androstendione, dehydroepiandrosterone sulfate). Buechler et al. [15] found that insulin

Abbreviations: RARRES2, retinoic acid receptor responder protein 2; TIG2, tazarotene induced gene 2 protein; CMKLR1, chemokine like receptor 1; TNF-α, tumor necrosis factor-α; WAT, white adipose tissue; BMI, body mass index; ACLY, ATP-citrate lyase.

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increased chemerin release from human adipocytes, while cellular chemerin levels were not affected. It has been shown that in 3T3-L1 cells, interleukin-1β increases chemerin mRNA level and chemerin secretion into medium [16]. TNF-α appears to be another factor stimulating chemerin production by 3T3-L1 cells and primary adipocytes, but not in liver [17]. In *db/db* mice chemerin gene expression is down-regulated in epididymal adipose tissue and was not found to be changed in the liver [8].

Chemerin gene expression is up-regulated by high fat diet in mouse adipose tissue [7]. In normal glucose tolerance animals (*Psammomys obesus*), fasting was without effect on chemerin gene expression in mesenteric adipose tissue; however, in impaired glucose tolerant and diabetic animals, fasting caused an increase in chemerin gene expression in mesenteric adipose tissue [5]. Altogether, these results suggest that chemerin gene expression is potentially regulated by nutrient intake. However, there is no data available on the effect of food restriction and food restriction–refeeding on chemerin gene expression. Understanding the effects of food restriction and food restriction–refeeding on chemerin gene expression in adipose tissue depots is crucial because food restriction is an important part of insulin resistance and type 2 diabetes prevention as well as weight loss regimens [18]. During preparation of this paper, Chakaroun et al. [19] reported that weight loss and exercise might regulate chemerin gene expression in human adipose tissue. The aim of the present study was to elucidate the effect of nutritional status, especially food restriction and food restriction–refeeding on chemerin gene expression in rat white adipose tissue (WAT) and liver and on circulating chemerin concentration.

2. Materials and methods

2.1. Prolonged food restriction experiment

Ten-week-old male Wistar rats, weighing on average 245 g at the onset of the experiment, housed in individual wire-mesh cages, were maintained at 22 °C under a light to dark (12 h:12 h) cycle with lights on at 7:00 a.m. The animals were treated as described recently by Turyn et al. [20]. Briefly, the rats were randomly divided into two groups. Control animals (n = 10) were allowed free access to food and tap water. The animals in the remaining group (n = 20) were allowed free access to tap water and were provided 50% of the total amount of food consumed by the control group. The food was replenished every day 2 h before the lights off period. The majority of food supplied was consumed within the first 2 h of access. Thus, in contrast to the control (*ad libitum* fed rats), rats maintained on restricted diet ate when the food was available regardless of the light cycle. An average daily food intake by the control group was measured by the difference in weight between the amount of food provided and the amount of food remaining over a period of one day. The average daily food intake by the control rats was approximately 26 g throughout the duration of the experiment. The commercial diet used in all groups was the same as described by Turyn et al. [20]. After one month of treatment, the rats kept on restricted diet were randomly divided into two subgroups: a) 10 rats that were kept for an additional two days on a restricted diet, and b) 10 rats that were fed *ad libitum* for two days.

2.2. Short-term fasting–refeeding experiment

Three-month-old male Wistar rats, weighing on average 320 g, housed in individual wire-mesh cages were maintained at 22 °C under a light:dark (12 h:12 h) cycle with lights on at 7:00 a.m. The animals were treated as described recently by Turyn et al. [20]. Briefly, the rats were divided randomly into three groups: a) control animals (n = 10) were allowed free access to food and tap water; b) fasted for 72 h animals (n = 10); and c) fasted for 72 h and then refed *ad libitum* for 48 h animals (n = 10). The study was approved by the Local Ethics Committee for Experimental Animals in Gdansk, Poland, and was performed in accordance with the EU Directive 2010/63/EU for animal experiments.

2.3. Tissue collection

After the treatment, the rats were killed by decapitation under ketamine anesthesia (between 8:00 and 10:00 a.m.). Perirenal, epididymal, and inguinal WAT and liver were collected, weighted and rapidly frozen in liquid nitrogen for subsequent analyses of gene expression. The tissues were stored at −80 °C until further analysis was performed.

2.4. Isolation and primary culture of rat adipocytes

Adipocytes from epididymal WAT of ten-week-old, fed *ad libitum* (n = 10) or fasted for 24 h before the tissue collection (n = 10), male rats, were isolated by collagenase digestion as described by Rodbell [21]. The tissue was placed in polypropylene tubes (BD Falcon) with Krebs–Ringer buffer (37 °C), containing 1% bovine serum albumin, 5.5 mM glucose, 20 mM HEPES pH 7.6 and 1 mg/ml collagenase (type II; Sigma, USA). The tissue was finely cut with scissors and incubated for 1 h at 37 °C with continuous shaking. After incubation, the tissue was filtered through 180 μm nylon Millipore filters. The adipocytes were washed three times with Krebs–Ringer buffer. The adipocytes isolated from one rat were divided equally into four portions and transferred to incubation plate (Corning, USA) containing 2 ml Dulbecco's modified Eagle's medium (DMEM) with 5.5 mM glucose supplemented with 1% bovine serum albumin. The adipocytes were incubated for 6 or 24 h at 37 °C, 5% CO_2, one portion from each rat in the absence of insulin and the other portions in the presence of 1, 10 or 100 nM insulin (Sigma, USA).

2.5. RNA isolation

Total cellular RNA was extracted from frozen tissue or adipocytes by a guanidinium isothiocyanate–phenol/chloroform method [22]. The RNA concentration was determined from the absorbance at 260 nm. All samples had 260/280 nm absorbance ratio of about 2.0.

2.6. cDNA synthesis

First strand cDNA was synthesized from 4 μg of total RNA (RevertAid™ First Strand cDNA Synthesis Kit — Fermentas UAB, Lithuania). Prior to the amplification of cDNA, each RNA sample was treated with RNase-free DNase I (Fermentas UAB, Lithuania) at 37 °C for 30 min.

2.7. Determination of mRNA level

Chemerin and ATP-citrate lyase (ACLY), leptin, adiponectin, TNFα and SREBP-2 mRNA levels were quantified by real-time PCR using Chromo4 Real Time Detection System (Bio-Rad Laboratories, Inc., USA). Primers were designed with Sequence Analysis software package (Informagen, Newington, USA) from gene sequence obtained from Ensembl Genome Browser (www.ensembl.org). The following oligonucleotides primers pairs were used: 5′-GTGGACAGTGCTGAT GACCTGTTCTT-3′ (sense) and 5′-AGGCATTTCCGCTTCCTCCCATT-3′ (antisense) for chemerin; 5′-CTCACACGGAAGCTCATCAA-3′ (sense) and 5′-ATGGCAACACCCTCGTAGAC-3′ (antisense) for ACLY, ATCAAG ACCATTGTCACCAGGATC-3′ (sense) and 5′-CTGGTCCATCTTGGACAAA CTCA-3′ (antisense) for leptin; 5′-GAGAGAAGGGAGACGCAGGT-3′ (sense) and 5′-GAACATTGGGGACAGTGACG-3′ (antisense) for adiponectin; 5′-GCCACCATGAGCACGGAAAGCA-3′ (sense) and 5′-CCCGC CACGAGCAGGAATGAG-3′ (antisense) for TNFα; 5′-ACTGTCACTGGA GTCAGGTT-3′ (sense) and 5′-GACCAACAGCTTCACGAAGA-3′ (antisense)