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Metabolism Clinical and Experimental 55 (2006) 555–559

Metabolism
Clinical and Experimental

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T cell–mediated hepatic inflammation modulates adiponectin levels in mice: role of tumor necrosis factor α

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Received 13 May 2005; accepted 2 November 2005

Abstract

Experimental T cell–mediated hepatitis induced by concanavalin A (ConA) results in the initiation of an inflammatory response and the production of cytokines. Adiponectin is an adipocytokine produced by adipose tissue that is involved in the reciprocal regulation of other cytokines, including tumor necrosis factor α (TNF-α). Concanavalin A administration to C57BL/6J mice reduced circulating levels of adiponectin, whereas leptin was markedly increased. Adiponectin messenger RNA expression in adipose tissue was also decreased; however, the expression of both the adiponectin receptors remained unchanged. Neutralization of TNF-α reduced ConA-induced liver damage, and this was associated with restored circulating levels of adiponectin. These findings indicate that inflammation-induced TNF-α is a critical mediator of adipose-tissue-derived adiponectin in vivo.

1. Introduction

Adipocytokines play an important role in the regulation of metabolic and inflammatory responses [1,2]. Adiponectin is the most abundant circulating adipocytokine and is an important regulator of insulin sensitivity [3]. Several cell types, including macrophages, express adiponectin receptors [4]. Adiponectin directly affects the inflammatory response by regulating cytokine production and activity [5-8] and can also act as an antiapoptotic agent in a variety of cell types [9-11]. Adiponectin plays a protective role in both acute and chronic inflammatory liver disease in vivo in mice [8,12-14]. Reciprocal regulation between adiponectin and tumor necrosis factor α (TNF-α) has been demonstrated. In fact, although adiponectin inhibits TNF-α production and activity, TNF-α can suppress adiponectin production by adipocytes in vitro [8,12,15,16]. However, TNF-α can also act on a different cell type, the myocyte, to increase adiponectin expression both in vitro and in vivo [17]. Therefore, the end result of acute inflammation on circulating adiponectin levels is, to our best knowledge, still unknown.

The model of T lymphocyte–mediated hepatitis induced by administration of the T-cell mitogen concanavalin A (ConA) was used to evaluate whether acute inflammation modulates adiponectin levels in vivo and the possible role played by TNF-α [18,19]. In this system, the adipocytokines leptin and adiponectin both contribute to disease modulation by playing contrasting roles [8]. Cytokines, particularly TNF-α, play a crucial role in ConA-induced liver damage [20,21].

The current report demonstrates that acute hepatic inflammation induced by ConA leads to a significant reduction in adipose tissue–derived adiponectin and that ConA-induced TNF-α is a critical mediator of this effect.

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doi:10.1016/j.metabol.2005.11.008

2. Materials and methods

2.1. Mice

Animal protocols were approved by the animal studies committee of the University of Colorado Health Sciences Center. C57BL/6J mice were obtained from the Jackson Laboratories (Bar Harbor, ME).

2.2. Administration of ConA

ConA (Type IV-S, Sigma, S. Louis, MO) was injected intravenously in the tail vein at a dose of 200 μg per mouse, as previously described [22]. Mice were killed by cervical dislocation under isoflurane anesthesia 24 hours after administration of ConA or vehicle for evaluation of adiponectin and leptin levels. During this period, food was removed because adiponectin receptor expression is altered postprandially [23] and to eliminate the possible effects of ConA-induced anorexia. For neutralization of TNF-α activity, sTNFRp55 (Amgen, Thousands Oaks, CA) was administered intraperitoneally 1 hour before ConA injection at a dose of 200 μg per mouse.

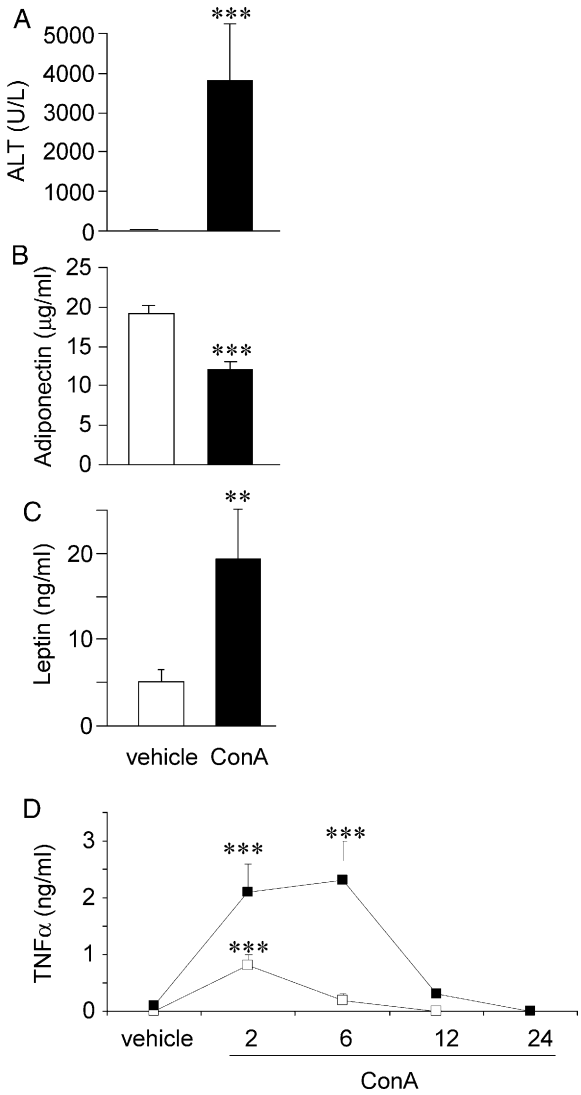

Fig. 1. Alanine aminotransferase, adiponectin, leptin, and TNF-α levels in ConA-injected mice. Alanine aminotransferase (A), adiponectin (B), and leptin (C) levels were measured in the serum of mice 24 hours after injection of either vehicle or ConA. Panel D shows the kinetics of serum (open squares) and liver-associated (closed squares) TNF-α levels after ConA administration. Data are expressed as mean ± SEM (n = 5 mice per group). $^{**}P < .01$, $^{***}P < .001$ vs vehicle by 2-tailed Student t test.

Table 1
Percentage distribution of HMW, MMW, and LMW adiponectin in vehicle and ConA-injected mice

	Vehicle	ConA
% HMW	27.6 ± 3.5	28.4 ± 4.2
% MMW	55.6 ± 7.6	56.4 ± 11.2
% LMW	16.8 ± 3.0	15.1 ± 5.1

Data are expressed as mean ± SEM of 3 mice per group. Serum obtained 24 hours after injection of either vehicle or ConA was fractionated over a HiLoad 16/60 Superdex 200 column, and the amount of adiponectin present in each fraction was evaluated by ELISA. HMW indicates high molecular weight; MMW, middle molecular weight, and LMW, low molecular weight.

2.3. Leptin, adiponectin, and TNF-α measurement

Leptin and TNF-α levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN). Adiponectin levels were assessed by an RIA kit (Linco Research, St Charles, MO). Serum fractionation was performed as previously described [24]. Briefly, 15 μL of serum was diluted in 0.5 mL of phosphate-buffered saline and fractionated over a HiLoad 16/60 Superdex 200 column (Amersham, Piscataway, NJ). Fractions (1 mL) were collected and assayed, using an ELISA kit, for adiponectin content.

2.4. Reverse transcriptase–polymerase chain reaction analysis of messenger RNA for identification of adiponectin and its receptors

Total messenger RNA (mRNA) was extracted from skeletal muscle (gastrocnemius), white adipose tissue (WAT) (parametrial), and liver. One microgram was reverse transcribed and subjected to duplex polymerase chain reaction amplification using the following primers: Adiponectin, forward 5′-GGA ACT TGT GCA GGT TGG A-3′T and reverse 5′-CGA ATG GGT ACA TTG GGA AC-3′; Adipo-R1, forward 5′-ACG TTG GAG AGT CAT CCC GTA T-3′ and reverse 5′-AGT GTG GAA GAG CCA GGA GA-3′; Adipo-R2, forward 5′-TCC CAG GAA GAT GAA GGG TTT AT-3′ and reverse 5′-CCA TGA AAA GGA AAG GCA GA-3′ and a competimer/primer mix specific for 18S rRNA (QuantumRNA kit; Ambion, Austin, TX). Each polymerase chain reaction was performed in duplicate on 2 individual preparations of reverse-transcribed cDNA.

2.5. Statistical analysis

Data are expressed as mean ± SEM. Statistical significance of differences between treatment and control groups

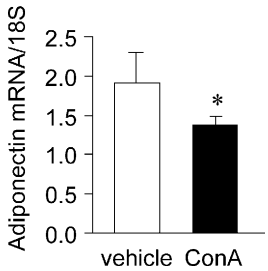

Fig. 2. Adipose tissue adiponectin mRNA expression. Adiponectin expression was measured in RNA extracted from WAT 24 hours after injection of either vehicle or ConA. Data are expressed as the ratio of adiponectin mRNA to 18S RNA and are expressed as mean ± SEM (n = 5 mice per group). *$P < .05$, vehicle by 2-tailed Student t test.

was determined by factorial analysis of variance. Statistical analyses were performed using the XLStat software (Addinsoft, Brooklyn, NY).

3. Results and discussion

As expected, administration of ConA induced liver damage, evaluated by increased serum alanine aminotransferase (ALT) levels 24 hours after treatment (Fig. 1A). At the same time point, serum adiponectin levels were significantly reduced (Fig. 1B), whereas serum leptin levels were significantly increased (Fig. 1C). As expected [25], ConA also induced a marked increase in serum and hepatic TNF-α levels (Fig. 1D). Previous reports had indicated that inflammation induced by a variety of stimuli up-regulates circulating leptin levels [26]. The current data demonstrate that ConA-induced hepatitis is also associated with high-circulating leptin. In contrast to leptin, serum adiponectin levels were significantly reduced after administration of ConA. These data support the notion that inflammation is a negative regulator of adiponectin production [27,28] and demonstrate that acute hepatic inflammation has a significant impact on circulating adiponectin levels. Adiponectin is present in the circulation in different polymerization states. The percentage of adiponectin present as high-molecular-weight complexes appears to play a protective role against insulin resistance [29]. Concanavalin A administration, despite significantly reducing total serum adiponectin levels, did not significantly alter the distribution of high- vs low-molecular-weight adiponectin. In fact, as indicated in Table 1, serum fractionation demonstrated that the percentage distribution of adiponectin did not significantly differ between control and ConA-injected mice.

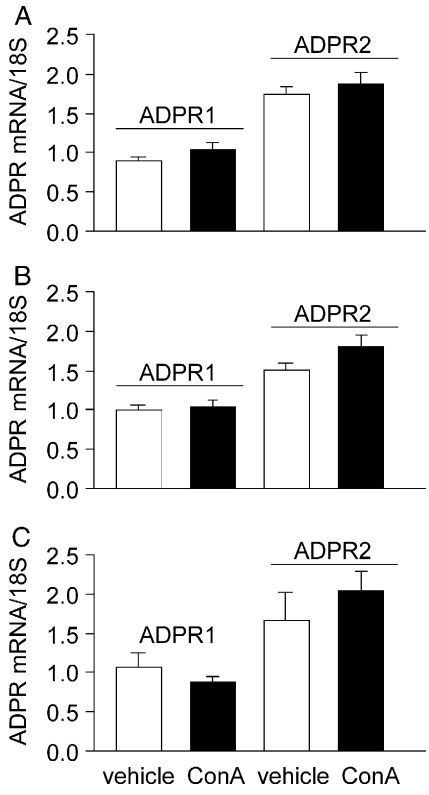

Fig. 3. Adipose tissue (A), muscle (B), and liver (C) adiponectin receptor 1 and 2 mRNA expression. Expression of adiponectin receptors 1 and 2 was measured in RNA extracted from WAT, muscle, and liver 24 hours after injection of either vehicle or ConA. Data are expressed as the ratio of adiponectin mRNA to 18S RNA and are expressed as mean ± SEM (n = 5 mice per group).

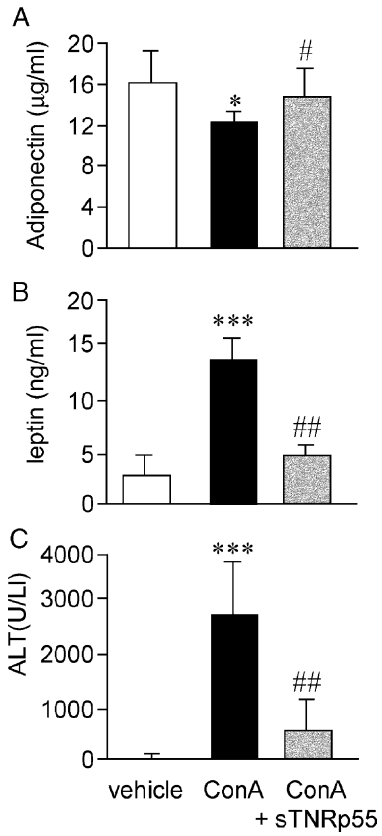

Fig. 4. Role of TNF-α in the modulation of adipokine and ALT levels by ConA. Serum adiponectin (A), leptin (B), and ALT (C) levels were measured in the serum of mice 24 hours after injection of either vehicle or ConA. For TNF-α neutralization, mice received a single intraperitoneal injection of sTNRp55 1 hour before ConA. Data are mean ± SEM (n = 10 mice per group). *$P < .05$, ***$P < .001$ vs vehicle; $^{\#}P < .05$, $^{\#\#}P < .01$ vs ConA by analysis of variance.

To evaluate whether reduced serum adiponectin is associated with a diminished expression of this adipocytokine in adipose tissue, we evaluated the adiponectin mRNA levels in adipose tissue homogenates in vehicle- and ConA-injected mice. As shown in Fig. 2, a 26% reduction in adiponectin mRNA expression was observed in adipose tissue obtained 24 hours after ConA compared with vehicle-injected mice. These results indicate that suppression of adipose tissue adiponectin production is a likely cause for the reduced circulating adiponectin levels observed after ConA administration.

In contrast to adiponectin mRNA expression, mRNA levels of the adiponectin receptor types 1 and 2 (AdipoR1 and AdipoR2) were not significantly altered in either adipose tissue, muscle, or liver (Fig. 3) 24 hours after administration of ConA. As mentioned above, TNF-α inhibits adiponectin expression by adipocytes in vitro [15,16] and is a pivotal mediator of ConA-induced hepatotoxicity [20,21]. Tumor necrosis factor α activity was neutralized by administration of soluble TNF receptors in ConA-injected mice to evaluate whether TNF-α mediates inhibition of adiponectin production after ConA. As shown in Fig. 4A, neutralization of TNF-α activity reversed the reduction in serum adiponectin levels induced by ConA. The reduced expression of adiponectin mRNA in adipose tissue induced by ConA was also reversed by neutralization of TNF-α (data not shown). Furthermore, the effectiveness of TNF-α neutralization was demonstrated by hepatoprotection, as evaluated by serum ALT levels, and by prevention of the increase in circulating leptin levels (Fig. 4B and C). These data confirm in vitro results demonstrating that TNF-α is an important inhibitor of adiponectin production in adipose tissue. Furthermore, despite evidence that TNF-α can induce adiponectin production in muscle [17], the net result of TNF-α in vivo is to lead to decreased circulating and adipose tissue–associated adiponectin levels. This is likely a consequence of the quantitatively predominant role played by adipose tissue—rather than muscle—in determining circulating adiponectin levels. However, our data do not exclude the possibility that local adiponectin levels in muscle might be modulated in the opposite way compared with adipose tissue during ConA-induced inflammation.

In conclusion, the current report demonstrates that similar to what was observed during obesity-induced chronic inflammation, acute hepatic inflammation is associated with significantly reduced circulating, adipose tissue–derived adiponectin levels and that this effect is mediated by TNF-α. In light of the important role of adiponectin in regulating insulin sensitivity, the current data suggest a possible mechanism linking acute inflammation with development of insulin resistance.

Acknowledgment

This study was supported by NIH DK-061483 (to GF). AMM is supported by an American Diabetes Association mentor–based postdoctoral fellowship (PN9909-189). The authors thank Jennifer Yoon and Leslie Pulawa for assistance in extracting RNA.

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