

Alterations of the classic pathway of complement in adipose tissue of obesity and insulin resistance

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¹Department of Food Science and Nutrition and ²Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, St. Paul, Minnesota; and ³Experimental Diabetes, Metabolism, and Nutrition Section, Diabetes Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

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Zhang J, Wright W, Bernlohr DA, Cushman SW, Chen X. Alterations of the classic pathway of complement in adipose tissue of obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 292: E1433–E1440, 2007. First published January 23, 2007; doi:10.1152/ajpendo.00664.2006.—Adipose tissue inflammation has recently been linked to the pathogenesis of obesity and insulin resistance. C1 complex comprising three distinct proteins, C1q, C1r, and C1s, involves the key initial activation of the classic pathway of complement and plays an important role in the initiation of inflammatory process. In this study, we investigated adipose expression and regulation of C1 complement subcomponents and C1 activation regulator decorin in obesity and insulin resistance. Expression of C1q in epididymal adipose tissue was increased consistently in *ob/ob* mice, Zucker obese rats, and high fat-diet-induced obese (HF-DIO) mice. Decorin was found to increase in expression in Zucker obese rats and HF-DIO mice but decrease in *ob/ob* mice. After TZD administration, C1q and decorin expression was reversed in Zucker obese rats and HF-DIO mice. Increased expression of C1 complement and decorin was observed in both primary adipose and stromal vascular cells isolated from Zucker obese rats. Upregulation of C1r and C1s expression was also perceived in adipose cells from insulin-resistant humans. Furthermore, expression of C1 complement and decorin is dysregulated in TNF- α -induced insulin resistance in 3T3-L1 adipocytes and cultured rat adipose cells as they become insulin resistant after 24-h culture. These data suggests that both adipose and immune cells are the sources for abnormal adipose tissue production of C1 complement and decorin in obesity. Our findings also demonstrate that excessive activation of the classic pathway of complement commonly occurs in obesity, suggesting its possible role in adipose tissue inflammation and insulin resistance.

C1 complement; gene expression

ADIPOSE TISSUE INFLAMMATION has recently been linked to obesity and insulin resistance (4, 35, 37, 44). Increasing fat mass results in the recruitment of immune cells such as macrophages to adipose tissue (43, 46). Both adipose and recruited immune cells in adipose tissue produce inflammatory cytokines and chemokines (21, 24, 26, 28) and participate in the inflammation process. The inflammatory cytokines TNF- α and IL-6 mainly target adipocytes and induce insulin resistance and alterations in glucose and lipid metabolism (2, 20, 32, 40). Improvement in insulin sensitivity with antidiabetic drugs such as thiazolidinedione (TZD) is associated with its anti-inflammatory action (14). Thus, inflammation seems to be at the root of insulin resistance in obesity and type 2 diabetes, and the ability

to reduce inflammation would be a positive feature of therapeutic intervention aimed at improving insulin resistance.

The complement system plays an essential role in the initiation and maintenance of inflammation, and its activation is launched via three different pathways: the classic pathway, the alternative pathway, and the lectin pathway (12, 22). A cascade of the alternative pathway activation is composed of complement C3, factors B and D. It has been found that adipose tissue is the main source of complement C3 and factor B/D (9), and adipocytes express and secrete complement C3 and factor B/D (8, 30). More importantly, studies demonstrated that expression of factor D (adipsin) gene is defective in several genetic models of rodent obesity (31). In a population-based cohort study (11), a strong correlation between plasma levels of C3, insulin, and glucose has been documented, indicating that C3 could serve as a risky factor for the development of insulin resistance and diabetes. Acylation-stimulating protein, which has a sequence identity with C3a, a cleavage fragment of C3, has been shown (3, 10, 16, 25) to stimulate triglyceride synthesis and glucose uptake in adipocytes. All of the data are indicative of the possible role of alternative pathway of complement in insulin resistance and obesity.

Activation of the classic pathway triggers an enzymatic cascade involving complement C1, C4, C2, and C3. C1 is a calcium-dependent complex comprising three distinct proteins: C1q, C1r, and C1s. C1 complex involves the key initial activation of the classic pathway of complement. The binding of C1 to classic pathway activators, primarily antigen-antibody complexes containing IgM, IgG1, and IgG3, initiates the activation of the classic pathway. Studies (41) have shown that the binding of C1q to its receptor on endothelial cells promotes the production of inflammatory cytokines of IL-8, IL-6, and MCP-1. Evidence from the other studies (1, 17) supports the notion that the activation of the classic pathway of complement involves the inflammatory process. Interestingly, the activation of classic pathway is regulated by other activators/inhibitors besides immunoglobulins. Earlier studies in immune-reactive tissues have reported that extracellular matrix proteins involve the activation of the classic pathway of complement and subsequently trigger inflammation. These proteins include decorin, biglycan, asporin, fibromodulin, lumican, proline- and arginine-rich end leucine-rich repeat protein, and chondroadherin (19), fibronectin (5, 39), and laminin (6). Fibromodulin directly binds to C1q, leading to the activation of the classic pathway (38). In contrast, decorin and biglycan bind to C1q as

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inhibitors, resulting in the suppression of inflammatory cytokine production and inflammatory responses (18). More attractively, C-reactive protein (CRP), a biomarker for inflammation and prediction of the risk for metabolic syndrome, has recently been demonstrated to be able to bind to C1q, serving as a classic pathway activator (27).

The above information together suggests that the activation of complement pathways may play a key role in inflammation and the pathogenesis of obesity and insulin resistance. However, it has not been reported how activation of the classic pathway of complement in adipose tissue is linked to obesity and insulin resistance. To gain insight into the possible role of the activation of the classic pathway of complement in adipocyte biology and insulin resistance, we examined the expression of C1 complement subcomponents and decorin, the C1 complement activation regulator, in adipose cells and its regulation in obesity and insulin resistance. We have demonstrated that adipocytes express C1 complement components and decorin. The expression level of C1 complement components significantly changed in adipose cells induced to insulin resistance *in vitro* and in adipose tissue in several animal models of obesity and insulin-resistant subjects.

MATERIALS AND METHODS

Animals

National Institutes of Health guidelines for animal handling were followed in this study, and experimental procedures were approved by animal care and use committee at the University of Minnesota.

Mice. Male C57BL/BJ and *ob/ob* mice used in this study were obtained from Jackson Laboratory (Bar Harbor, ME). At 3 wk of age, C57BL/BJ mice were on a high-fat diet (20% protein, 35.5% fat, 36.3% carbohydrate; Bioserve, Frenchtown, NJ) and maintained at 70°F on a 12:12-h light-dark cycle and fed *ad libitum*. At 12 wk of age, animals were then on high-fat diet containing 2% nonfat powdered milk with or without 0.2% troglitazone (Park Davis, Ann Arbor, MI) for 14 days. For the fasting study, mice at 9–10 wk of age were fasted for 24 h or fasted for 24 h and then refed a normal chow diet for 24 h. At the end of experiments animals were euthanized, and adipose tissue samples were removed for RNA extraction.

Rats. Rats used in this study include 5- to 6-wk-old male Sprague-Dawley rats (CD strain) and 7- to 8-wk-old lean and Zucker obese rats (Charles River Laboratories, Wilmington, MA). Rats were on the normal rodent diet during the experiments. Zucker obese rats (3 rats/group) were treated with rosiglitazone (3 mg/kg body wt) via gavage for 12 days. Three independent experiments were performed. At the end of experiments rats were euthanized, adipose tissue from epididymal fat pads was removed, and adipose cells were isolated.

Cell Cultures

3T3-L1 cell cultures. 3T3-L1 cells were grown in Dulbecco's modified Eagle's medium with 100 IU/ml penicillin/streptomycin (PEST) and 10% bovine calf serum until confluent. Two days after confluence, cells were then differentiated with the differentiation cocktail (Dulbecco's modified Eagle's medium with 100 IU/ml PEST and 10% fetal bovine serum, 500 μ M 3-isobutyl-1-methylxanthine, 1 μ g/ml insulin, and 100 ng/ml dexamethasone) for 2 days, as described (34). The cultures were then continued with Dulbecco's modified Eagle's medium (100 IU/ml PEST, 10% fetal bovine serum, and 1 μ g/ml insulin) for 6 days. On *day 8* of differentiation, medium was changed to 0.5% fetal bovine serum overnight. The cells were then washed with PBS and incubated with or without 3 nM TNF- α or 1 μ M rosiglitazone in Dulbecco's modified Eagle's medium containing

0.5% bovine serum albumin for different times, as indicated in RESULTS.

Primary rat adipose cell isolation and culture. Preparation of isolated rat epididymal adipose cells from normal male rats and lean and obese Zucker rats was performed as described previously (42). Isolated cells were washed twice with Dulbecco's modified Eagle's medium containing 25 mM glucose, 25 mM HEPES, 4 mM L-glutamine, 200 nM *N*⁶-(2-phenylisopropyl)adenosine, and 75 μ g/ml gentamycin and resuspended to a cytocrit of 40% ($5-6 \times 10^6$ cells/ml). After final wash, adipose and stromal vascular (S-V) cells from lean and obese Zucker rats were immediately frozen in liquid nitrogen for RNA extraction. Adipose cells from normal rats were cultured for 24 h at 37°C, 5% CO₂ in Dulbecco's modified Eagle's medium containing 3.5% bovine serum albumin. Then they were harvested for mRNA extraction.

Quantitative Real-Time RT-PCR

Total RNAs from isolated rat S-V and adipose cells from epididymal fat depot and adipose tissue were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA was treated with RQ1 DNase (Promega, Madison, WI) at 37°C for 30 min, followed by extraction with phenol-chloroform and ethanol precipitation. The first-strand cDNA was generated using the oligo(dT) primer in the first-strand cDNA synthesis kit (Promega). One microliter of the reverse transcription reaction mix was amplified with primers specific for genes listed in Table 1 by 30–35 cycles, 94°C for 1 min, 53°C for 1 min, and 72°C for 5 min. For real-time PCR, samples were subsequently diluted 1:20, and 10 μ l of cDNA were used in each 25- μ l real-time PCR reaction using the SYBR GreenER qPCR SuperMix Universal (Invitrogen) at ABI 7500 by Absolute Quantation. Results of qPCR assays were analyzed using Analysis software supplied with the ABI 7500 system. A standard curve for each primer set was generated in each assay and used to derive the copy number of target sequences in unknown samples. β -Actin was selected as an internal standard, and all the raw data were expressed as the ratio of the copy number of selected gene to β -actin. Statistical significance was determined by an unpaired, two-tailed Student's *t*-test.

cDNA Microarray

Microarray fabrication. Mouse cDNA microarrays with a total of 10,816 elements were printed on poly-L-lysine-coated slides. Mouse cDNA microarrays can be used for rat studies, as indicated in a previous study (29). The cDNAs used were provided by Dr. Bento Soares, University of Iowa. Plasmids were extracted from the bacteria using QiaPrep turbo kits and a BioRobot 8000 (Qiagen, Valencia, CA). The cDNA inserts were amplified with modified M13 primers (M13F 5'-GTTGTAAAACGACGGCCAGTG-3' and M13R 5'-CACAGGAAACAGCTATG-3') and purified with MultiScreen PCR plates (Millipore, Billerica, MA). Detailed descriptions of coating glass with poly-L-lysine and postprocessing the printed slides are available at <http://cmgm.stanford.edu/pbrown/mguide/index.html>.

RNA extraction and labeling. Total RNAs from isolated rat adipose cells were extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. In this study, three independent experiments per time point and three cDNA microarrays per RNA sample were used. For each experiment, total RNA of cultured cells from 6 to 8 normal rats or fresh isolated adipose cells from three lean and obese Zucker rats were pooled and subjected to subsequent cDNA synthesis, labeling, hybridization, and analysis. First- and second-strand cDNA syntheses, labeling, hybridization, and washing were performed as described (45).

Array scanning and data analysis. The arrays were scanned with a GenePix 4000A scanner (Molecular Devices) at 10- μ m resolution. The photomultiplier tube voltage settings were varied to obtain the maximum signal intensities with <1% probe saturation. The resulting

Table 1. Sequences of primers for real-time RT-PCR

Accession	Gene	Primer Sequence	
		Forward	Reverse
AK145308	β -Actin	5'-CCTAAGGCCAACCGTGAAAA-3'	5'-GAGGCATACAGGGACAGCACA-3'
AY754346	Adiponectin	5'-GCAGAGATGGCACTCCTGGA-3'	5'-CCCTTCAGCTCCTGTCTATCC-3'
NM_007988	FAS	5'-CTGGACTCGCTCATGGGTG-3'	5'-CATTCCCTGAAGTTCCGGCAG-3'
BC075688	F4/80	5'-GAAGGCTCCCAAGGATATGGA-3'	5'-TGCTTGGCATTGCTGTATCTG-3'
BC024384	IL-18	5'-TCTTCTGCAACCTCCAGCATC-3'	5'-GACATGGCAGCCATTGTTCC-3'
NM_011333	MCP-1	5'-CTTCTGGGCTGCTGTTCA-3'	5'-GAGTAGCAGCAGGTGAGTGGG-3'
BC002086	C1q	5'-GGCTGGAGCATCCAGTTTGA-3'	5'-GTCATGGTCAGCACACAGGC-3'
AF459018	C1r	5'-TTCATGGAGGGAATCAGGC-3'	5'-TGATCTTGCACCTGGGCAT-3'
BC111880	C1s	5'-CCAGAGCAATACTCTCGGCAT-3'	5'-TTTGGCACAGGAGATGGG-3'
BC060126	Decorin	5'-CCCTACCGATGCCAGTGCA-3'	5'-AGTTTGTCTCGGTGGAAAA-3'
NM_010570	IRS-1	5'-TCTCAACAGCAGTCCCTACC-3'	5'-GCACAATGTACAACGTGCAC-3'
BC014282	GLUT4	5'-CCATTCCCTGGTTCATTGTG-3'	5'-GTTTGGCCCTCAGTCATTC-3'
AY243585	PPAR γ	5'-ACCACTCGCATTCTTTGAC-3'	5'-AAGGCACCTCTGAAACCGAC-3'
NM_008493	Leptin	5'-GAAGCCTCACTCTACTCCAC-3'	5'-TCTACCCATCCCTACCTCAC-3'
DQ788722	IL-6	5'-TCTGCAAGACACTTCCATCC-3'	5'-TTAGCCACTCCTTCTGTGAC-3'

FAS, fatty acid synthase; MCP-1, monocyte chemoattractant protein-1; IRS-1, insulin receptor substrate-1; GLUT4, glucose transporter 4; PPAR γ , peroxisome proliferator-activated receptor- γ .

images were analyzed using IPLab (Scanalytics, Rockville, MD) and ArraySuite (National Human Genome Research Institute, Bethesda, MD) software. In all of the experiments described ratios were assigned quality scores (7, 45), and those with scores of <1 were removed from the data sets. Class comparison between red and green channels was performed using univariate paired *t*-test (with random variance model) to identify the differentially expressed genes that were statistically significant at a $P < 0.05$ level (mAdb Gateway and BRB Array Tools; <http://madb.nci.nih.gov>).

For the human studies, equal amounts (10 μ g from each individual) of RNA isolated from adipose cells of eight insulin-resistant or eight insulin-sensitive individuals were pooled to constitute two insulin-resistant and two insulin-sensitive adipocyte pools with four individuals in each pool. RNA samples were hybridized with Affymetrix GeneChip, as described in a previous study (47).

RESULTS

Expression of C1 Complement Subcomponents and Decorin in Adipose Tissue of Obesity and its Regulation by TZD

To investigate the changes in the classic pathway of complement and their correlation with insulin resistance in the pathophysiological states of obesity, we examined the expression of C1 complement subcomponents and decorin in adipose tissue in genetic (*ob/ob*) and high-fat diet-induced obese (HF-DIO) mice. In addition, TZD effect on the expression of C1 complement subcomponents and decorin in adipose tissue of HF-DIO mice was examined. Compared with nonobese control mice, expression of C1q and C1s was significantly ($P < 0.05$) increased in epididymal adipose tissue of *ob/ob* mice by ~ 11 - and 6-fold, respectively; decorin expression, however, was significantly ($P < 0.05$) decreased in *ob/ob* mice (Fig. 1A).

TZD effect on expression levels of C1 complement and decorin were examined in HF-DIO mice, since the model of diet-induced obesity is the closest in similarity to human obesity. The body weight of C57BL/BJ mice on a high-fat diet for 9 wk was 34.29 ± 3.05 g (mean \pm SE), significantly higher than that of mice fed a standard diet (22.85 ± 0.18 g; mean \pm SE, $P < 0.01$). As illustrated in Fig. 1B, expression of C1q and decorin was significantly ($P < 0.05$) elevated in epididymal fat pad of HF-DIO mice compared with nonobese control mice. As indicative effects of TZD, body weight gain in HF-DIO

mice after TZD administration for 14 days was significantly higher than that in control (HF-DIO) mice (TZD: 4.9 ± 2.3 g vs. control: 2.24 ± 2.33 g; mean \pm SE, $P < 0.01$). However, the differences in plasma levels of triglyceride and free fatty acid didn't reach the statistical significance (data not shown). Interestingly, 14-day treatment of TZD reversed the expression of C1q and decorin in adipose tissue of HF-DIO mice (Fig.

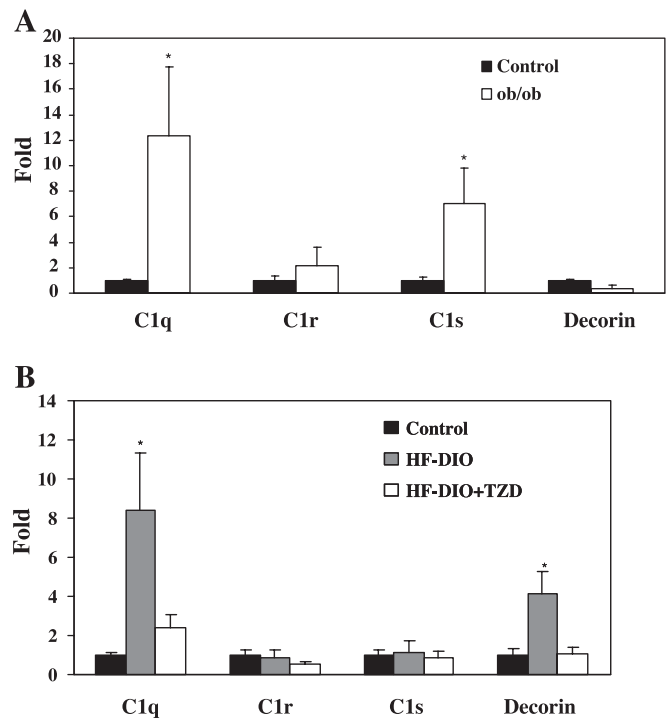


Fig. 1. Expression of C1 subcomponents and decorin in adipose tissue of *ob/ob* and high-fat diet-induced obese (HF-DIO) mice. Total RNA was extracted from epididymal fat pad of *ob/ob* mice (A) and HF-DIO mice treated with or without troglitazone for 14 days (B). Levels of expression for C1 subcomponents and decorin were detected by real-time RT-PCR. The expression levels in each sample were normalized to the level in lean control and shown as fold changes. The results are presented as means \pm SE. * $P < 0.05$ ($n = 16$, duplicates of 8 individual animals for each group). TZD, thiazolidinedione.

1B). This indicates that C1q and decorin are the possible early targeting genes of TZD effects.

Expression of C1 Complement Subcomponents and Decorin in Primary Adipose Cells Isolated From Insulin-Resistant Animals and Human Subjects

Since both immune and adipose cells possibly contribute to the adipose tissue production of C1 complement subcomponents, it is essential to identify the cellular source of their production, particularly in obese and insulin-resistant states. Table 2 illustrated levels of mRNA expression of C1 complement subcomponents and decorin detected by microarrays in primary adipose and S-V cells isolated from obese Zucker rats and insulin-resistant subjects. Compared with insulin-sensitive controls, expression of C1r and C1s consistently increased in adipose cells of obese Zucker rats and insulin-resistant humans (Table 2). Decorin expression was also increased in adipose cells of obese Zucker rats. In a comparison between S-V and adipose cells isolated from obese Zucker rats, levels of expression for C1q, C1r, C1s, and decorin were all increased in both S-V and adipose cells, but the magnitude of changes for C1q, C1r, C1s, and decorin appear to be higher in S-V cells (Table 2).

Microarray-based gene expression of C1 complement and decorin in Zucker obese rats was validated by the quantitative real-time RT-PCR. As shown in Fig. 2, the expression of C1q and decorin was concordant with array data; i.e., their mRNA levels were higher in both S-V (Fig. 2A) and adipose cells (Fig. 2B) from Zucker obese rats than that from lean rats. Inconsistent with array data, both C1r and C1s had increased expression level in S-V cells from Zucker obese vs. lean rats. Whereas C1r expression was decreased, C1s was not changed in adipose cells by quantitative real-time RT-PCR. The level of C1q expression was reduced in Zucker obese rats after 12 days of TZD administration, where a significant improvement of insulin sensitivity was observed (data not shown).

Expression of C1 Complement Subcomponents and Decorin in Cultured Adipocytes

The direct relationship between C1 complement expression and the development of insulin resistance in adipose cells was examined in cultured adipose cell model of insulin resistance. Our group and others (33) have demonstrated that primary isolated adipose cells spontaneously and gradually develop insulin resistance when cultured in vitro for 24 h. This insulin resistance may be triggered by the cytokines released from

adipose tissue during the collagenase digestion for the isolation of adipose cells. Thus, we investigated whether dysregulation of C1 complement expression is associated with cytokine-induced inflammatory response and insulin resistance in cultured adipose cells. Compared with fresh isolated and insulin-sensitive adipose cells from normal rats, cells have significantly decreased expression of C1q and increased expression of C1r, C1s, and decorin (Table 2) as they developed insulin resistance after 24 h in culture.

In addition, the expression of complement mRNAs in adipocytes and their relation to differentiation was examined in 3T3-L1 adipocytes. Total mRNA was extracted from 3T3-L1 cells at different stages of differentiation, and levels of mRNA expression of C1 complement subcomponents and decorin were examined by real-time RT-PCR. As shown in Fig. 3, all three subcomponents of complement 1 (C1q, C1r, and C1s) and decorin were expressed at a relatively higher level in undifferentiated preadipocytes (0 vs. 96 h: C1q, $P > 0.05$; C1r, $P < 0.01$; C1s, $P > 0.05$; and decorin, $P < 0.01$). Expression of these four genes was increased within the first 24 h of differentiation but declined upon full (8-day) differentiation (0 vs. 24 h: C1q, $P < 0.05$; C1r, $P > 0.05$; C1s, $P > 0.05$; and decorin, $P < 0.01$; $n = 9$, triplicates of 3 independent experiments).

To prove that adipocyte expression of mRNA for C1 complement subcomponents and decorin is dysregulated in insulin resistance, 3T3-L1 adipocytes were made insulin resistant by treatment with TNF- α . 3T3-L1 adipocytes after 8 days of differentiation were exposed to 0.5% bovine calf serum and 1 mg/ml glucose for overnight, followed by 3 nM TNF- α treatment for 24 h. TNF- α treatment resulted in decreased expression of mRNAs for insulin receptor substrate-1, glucose transporter 4, peroxisome proliferator-activated receptor- γ , and leptin and increased expression of IL-6 (data not shown), indicating that TNF- α -treated cells are insulin resistant. In these insulin-resistant adipocytes, expression of mRNA for C1s was significantly increased, whereas C1q expression was decreased (Fig. 4). C1s had a trend toward an increase in expression level, but decorin expression was not significantly changed (Fig. 4).

Expression of C1 Complement Subcomponents, Decorin, and Inflammation Markers in Adipose Tissue of Mice in Response to Fasting

It is known that metabolic defects and inflammation are clustered in insulin resistance and obesity. However, the inter-

Table 2. Expression of mRNA for C1 complement subcomponents and decorin in primary adipose cells

Accession	Gene	S-V Cells from Zucker Rats	Adipose Cells from Zucker Rats	Cultured Rat Adipose Cells	Human Adipose Cells
		Fold Changes (obese/lean)	Fold Changes (obese/lean)	Fold Changes (24-h/0-h culture)	Fold Changes (IR/IS)
NM_007572	Complement component 1, q subcomponent, α	2.32	1.28	0.54	NA
BC067001	Complement component 1, q subcomponent, β	1.53	1.78	0.42	NA
NM_007574	Complement component 1, q subcomponent, c	1.71	1.38	0.64	NA
BC093528	Complement component 1, r subcomponent	3.09	1.21	9.12	2.09
BC022123	Complement component 1, s subcomponent	2.48	1.24	5.77	1.91
NM_007833	Decorin	4.11	1.68	2.95	NA

S-V, stromal vascular; IR, insulin resistant; IS, insulin sensitive; NA, not applicable.

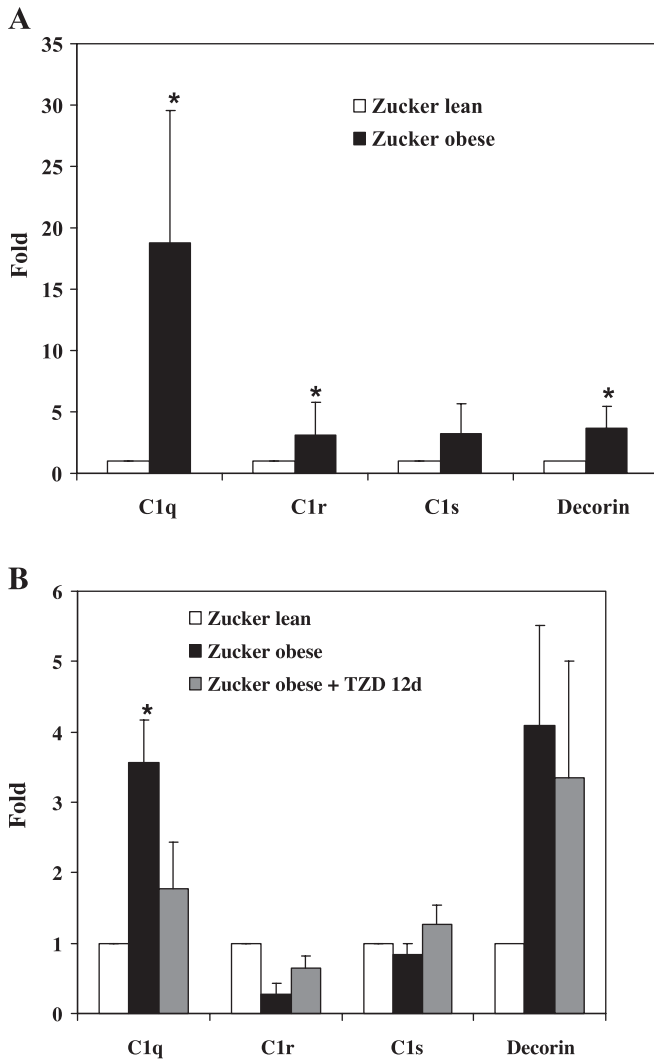


Fig. 2. Validation of microarray results by quantitative real-time PCR. An independent analysis was performed to confirm the expression of C1 subcomponents and decorin in primary stromal vascular (S-V; A) and adipose (B) cells isolated from Zucker lean and obese rats with or without 12-day TZD administration. The expression levels in each sample were normalized to the level in lean control and shown as fold changes. The results are presented as means \pm SE. * $P < 0.05$ ($n = 6$, duplicates of 3 individual animals for each group).

relation between metabolic and inflammatory responses and how these responses are interregulated in adipose tissue are not clear. To assess whether changes in C1 expression are in relation to metabolic alterations, we examined the effect of acute changes in nutritional status, i.e., fasting on the regulation of C1 expression in adipose tissue. In the fasting state, the body encounters many changes in both levels of hormones, mainly insulin and energy metabolism. Thus, fasting is a good approach for investigating whether C1 and inflammatory factors are regulated by changes in whole body metabolism. The expression of C1 was compared with that of metabolic gene fatty acid synthase (FAS) and inflammation markers [F4/80, monocyte chemoattractant protein-1 (MCP-1), IL-18]. As expected, levels of mRNA expression of FAS were decreased in epididymal adipose tissue of normal mice in response to 24-h fasting and recovered after 24-h refeeding (data not shown).

However, the levels of expression for inflammation markers of F4/80, MCP-1, and IL-18 were not changed regardless of fasting/refeeding (Fig. 5A). Similarly, fasting/refeeding did not result in the alteration in the expression of mRNA for C1q, C1r, C1s, and decorin genes (Fig. 5B).

DISCUSSION

The biological significance of complement system in adipose tissue has been speculated in the earlier studies (8). Several proximal components of alternative pathway of complement such as C3, factor D/adipsin, and factor B are expressed in adipose cells, and their expression is dysregulated in obesity and insulin resistance (9). Recently, an association of lectin pathway of complement with obesity and insulin resistance has also been demonstrated (13). Interestingly, the activation of alternative pathway of complement in adipocytes does not cause traditionally recognized immunological effects, such as cell lysis (8). The observation that adipose cells produce only proximal, but not terminal, components of complement such as C5 and C2 (8) suggests that the activation of complement system may lead to metabolic consequences through a different mechanism in adipose cells. This notion is supported by the following findings. For example, acylation-stimulating protein, a cleavage fragment of C3, affects glucose (25) and lipid metabolism (3, 10, 16) in human adipocytes. Besides, adiponectin, a well-known adipose-derived hormone, shares a structural similarity with C1q complement factor (36). But it is unclear whether the production of adiponectin is associated with the activation of the classic pathway of complement. Thus, this attractive hypothesis and notion drove us to investigate the classic pathway of complement in obesity and insulin resistance. Furthermore, the observations that CRP, decorin, and biglycan serve as regulators of the activation of the classic pathway make it more interesting to examine the changes in activation regulators (18, 23, 27). Although one study has shown that C1 complement expression is altered in omental adipose tissue of obese men (15), an in-depth investigation is essential for the complete understanding of the potential role of classic pathway of complement in adipose tissue metabolism and insulin resistance.

It is reasonable to hypothesize that the C1 complement system plays a role in the development of insulin resistance in obesity, since it involves the key part of the inflammatory

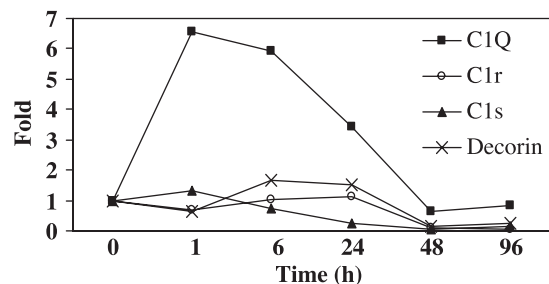


Fig. 3. Expression of mRNA for C1 subcomponents and decorin in 3T3-L1 adipocytes. Total RNA was extracted from 3T3-L1 adipocytes at the indicated times during the differentiation. Levels of mRNA expression were detected by real-time RT-PCR, as described in MATERIALS AND METHODS. The expression levels in each sample were normalized to the level in control (0 h) and shown as fold changes. The results represent 3 independent experiments of means \pm SE.

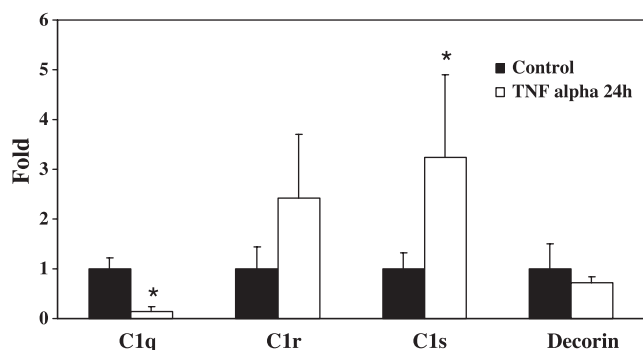


Fig. 4. Effect of TNF- α on mRNA expression of C1 complement and decorin in 3T3-L1 adipocytes. After 8 days of differentiation, 3T3-L1 adipocytes were treated with TNF- α for 24 h. Total RNA was extracted for levels of expression for insulin receptor substrate-1, glucose transporter 4, peroxisome proliferator-activated receptor- γ , leptin, and IL-6 (A) by RT-PCR and C1 complement subcomponents and decorin (B) by real-time RT-PCR. The expression levels in each sample were normalized to the level in untreated control and shown as fold changes. The results represent 3 independent experiments of means \pm SE.

process. To address this relationship between C1 complement system and insulin sensitivity, we investigated the expression of C1 complement and decorin and its regulation by TZD in several rodent models of obesity. Interestingly, we observed that the alterations in C1q expression were consistent across all three of the rodent models of insulin resistance that we examined. For example, its adipose expression was increased in *ob/ob* and HF-DIO mice and Zucker obese rats. TZD treatment caused the reversal of C1q expression in both Zucker obese rats and HF-DIO mice, suggesting that C1q is the key complement component that may play a crucial role in the classic pathway activation and inflammation. However, the expression of decorin is increased in HF-DIO mice but decreased in *ob/ob* mice, and C1s expression is not significantly changed in HF-DIO mice but increased in *ob/ob* mice. It is important to note that *ob/ob* and HF-DIO mice are at the different disease stages. For instance, HF-DIO mice are insulin resistant, but not diabetic, at an earlier stage of diabetes, whereas *ob/ob* mice are hyperglycemic and diabetic. Because decorin has been reported to act as an inhibitor of classic complement activation, increased decorin could be a protective response to the over-activation of complement pathway at the beginning of the disease development. It is possible that this response is lost with disease progression, whereas C1s expression increases at the late stage of disease. Thus, it is likely that levels of decorin and C1s expression are associated with the progression of diseases.

More strikingly, we observed that 14-day treatment of TZD reverses the expression of C1q and decorin in adipose tissue of HF-DIO mice prior to observable metabolic changes. Since weight gain and a trend of decrease in TG levels were observed in HF-DIO mice after TZD treatment (data not shown), it is likely that 14-day treatment may already cause crucial early changes at the level of gene expression, which is essential for the subsequent detectable metabolic changes. This suggests that changes in adipose expression of C1q complement and decorin are correlated with systemic insulin sensitivity. C1r and C1s, however, show less impact in terms of their changes in rodent models of obesity. Overall, our observations lead to a conclusion that upregulation of C1q complement in adipose

tissue appears to commonly occur in vivo in insulin resistance, suggesting that excessive activation of C1 complement in adipose tissue may occur in obesity and link to inflammation and insulin resistance. Changes in decorin expression may reflect the progression of insulin resistance and diabetes. Thus, control of the activation of C1 complement and/or its regulators could be potential therapeutic targets for insulin resistance.

Adipose tissue inflammation has been associated with obesity and insulin resistance. Increased numbers of macrophages and other immune cells contribute to increased adipose tissue production of inflammatory cytokines and its caused metabolic consequences. Thus, it is essential to understand which cellular source of C1 complement and decorin is dysregulated in obese states. To achieve this goal, macrophage-enriched S-V cells and adipose cells were isolated from Zucker rats, and expression of C1 complement and decorin was compared. Expression of C1 complement and decorin is upregulated in primary adipose and S-V cells isolated from obese Zucker rats. More interestingly, increased expression of C1r and C1s was also seen in primary adipose cells from abdominal subcutaneous adipose tissue of the patients with insulin resistance. As described in a previous study (47), these patients have normal body weight and BMI but significantly larger adipose cells and are more insulin resistant compared with normal subjects. The

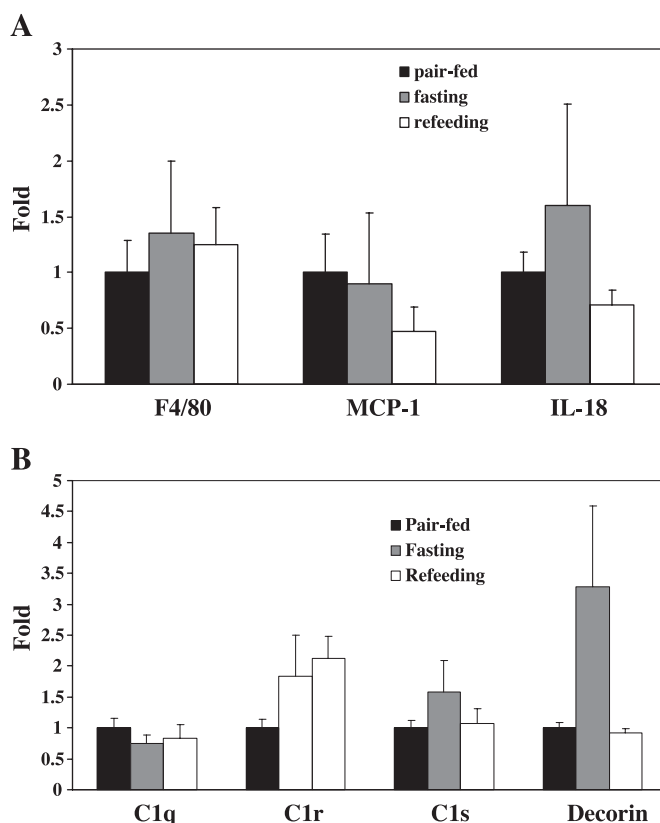


Fig. 5. Effects of nutritional status on expression of C1 subcomponents and decorin in adipose tissue of mice. Total RNA was extracted from normal mice fasted for 24 h and refed a diet for 24 h. Levels of expression for C1 subcomponents and decorin were detected by real-time RT-PCR. The expression levels in each sample were normalized to the level in control (pair fed) and shown as fold changes. The results are presented as means \pm SE. $P > 0.05$ ($n = 8$, duplicates of 4 individual animals for each group). MCP-1, monocyte chemoattractant protein-1.

above data suggest that both adipose and immune cells invaded into adipose tissue are the major source for abnormal adipose production of C1 complement and decorin in obesity and insulin resistance.

Our data from cultured adipocytes provide further evidence that adipose cells are also the major site of dysregulated C1 complement production, and adipocyte expression of C1 complement and decorin is associated with insulin resistance. We show that 3T3-L1 adipocytes express C1 complement at the detectable level, although the levels of expression were declined compared with undifferentiated preadipocytes. Interestingly, the adipocyte production of C1s and C1r complements is markedly upregulated when adipocytes are induced to become resistant to insulin by TNF- α treatment, whereas C1q is downregulated, which is opposite to what is observed in adipose tissue of obese animals. The similar phenomenon has also been observed in primary adipose cells when they develop into insulin resistance *in vitro*. For instance, primary adipose cells isolated from normal rats show a marked increase in expression of C1r, C1s, and decorin but a decrease in C1q expression when cultured *in vitro* for 24 h. The possible explanations for the discrepancy in C1q expression between cell culture and *in vivo* are as follows. As we observed, both adipose and immune cells in S-V fraction produce C1 complement and decorin, and their expression is dysregulated in both types of cells in obesity. Adipose tissue secretes a large number of adipokines and cytokines from both adipose and immune cells. These signaling molecules work in networks and coordinate the cross talk between adipose and S-V cells to maintain normal adipocyte functions and gene expression. It is evident that cultured adipocytes lost this networking or coordinating regulations, and TNF- α - or culturing-induced changes in C1q expression could not reflect the real alterations occurring in adipose tissue in which more complex interactions between different types of cells and networking effects of signaling molecules exist *in vivo*. Thus, C1q expression is more likely to be regulated cooperatively by multiple factors in several types of cells in adipose tissue. In addition, the development of inflammation in obesity could be a chronic process. Twenty-four-hour treatment *in vitro* may be a relatively short period of time for observing the changes in C1q expression and insulin resistance similarly occurring in obesity.

We hypothesized that abnormal production of C1 complement and other inflammatory factors in obesity develop through a chronic process, rather than an acute response to the metabolic regulation. Fasting, which induces acute metabolic changes, was used to test this hypothesis. Interestingly, 24-h fasting dramatically reduces expression of metabolic gene FAS, and refeeding restores the expression (data not shown). However, similar to F4/80, MCP-1, and IL-18, C1 complement expression is not changed in response to fasting/refeeding. These data support the notion that 1) changes in the regulation of C1 complement are the chronic process developed in obesity and 2) C1 complement or inflammation contributes to local rather than systemic regulation of metabolism.

In summary, our findings demonstrate that adipose expression of three key initial components of C1 complex as well as C1 activation regulator decorin are dysregulated in obesity and insulin resistance *in vivo* in several rodent models of obesity as well as human insulin resistance. Both adipose and immune cells present in S-V fraction are responsible for abnormal

production of C1 complement and decorin in obesity. Adipose expression of C1 and decorin is regulated depending upon insulin sensitivity. The development of abnormal production of C1 complement may be a chronic process. Thus, our findings provide the necessary information for further studying and understanding the role of adipose C1 complement activation system in obesity and insulin resistance. To achieve this goal, the following interesting questions need to be addressed. 1) What regulates adipose tissue C1 complement activation in obesity; 2) how is C1 complement activation in adipose tissue linked to insulin resistance; and 3) do unknown cleavage fragments resulting from C1 complement activation regulate adipocyte metabolism and play a role in the development of insulin resistance?

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