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Original Reports

A Correlative Relationship Between Chronic Pain and Insulin Resistance in Zucker Fatty Rats: Role of Downregulation of Insulin Receptors

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Abstract: **Epidemiological studies and meta-analyses report a strong relationship between chronic pain and abnormalities in glucose metabolism, but the exact relationship between chronic pain and insulin resistance in type 2 diabetes (T2D) remains unknown. Using a model of neuropathic thermal and tactile hypersensitivity induced by chronic constriction injury (CCI) of the sciatic nerve in Zucker Diabetic Fatty (ZDF) and Zucker Lean (ZL) littermates, we compared the recovery period of hypersensitivity and the progression of T2D and studied the possible involvement of insulin receptors (IRs) in the comorbidity of these 2 conditions. We found that the nociceptive thresholds to thermal and mechanical stimulation in naive ZDF rats were lower than in ZL littermates at 6 weeks of age. Although ZDF and ZL rats developed thermal and tactile hypersensitivity after CCI, it took a longer time nociceptive sensitivity to be restored in ZDF rats. Nerve injury accelerated the progression of T2D in ZDF rats, shown by an earlier onset of hyperglycemia, more severe hyperinsulinemia, and a higher concentration of glycosylated hemoglobin Alc 6 weeks after CCI, compared with those in naive ZDF and ZL rats. IR-immunoreactive cells were located across the central nervous system and skeletal muscles. In the central nervous system, IR coexpressed with a neuronal marker (neuronal nuclei) but not a glial marker (glial fibrillary acidic protein). There was a low level of IR expression in skeletal muscles of naive ZDF rats. In contrast, CCI reduced the IR expression in skeletal muscles as well as the ipsilateral spinal cord, primarily in the dorsal horn. In conclusion, our data suggest that the relationship between insulin resistance and chronic pain in ZDF rats is bidirectional and an impaired IR signaling system might be implicated in this reciprocal relationship.**

Perspective: ***Nerve injuries in genetically susceptible individuals might accelerate the development of insulin resistance as in T2D. A downregulated expression of IRs in the skeletal muscle innervated by the injured nerve is one of the underlying mechanisms.***

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Type 2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia and hyperinsulinemia. The incidence of obesity, insulin resistance, and T2D is increasing at an alarming rate and represents a significant clinical condition worldwide. T2D is frequently accompanied with painful diabetic neuropathy, among many other complications. Approximately 1 in 3 people with diabetes is affected by diabetic neuropathy, a major health problem that might present with excruciating pain and is responsible for substantial morbidity, increased mortality, and impaired quality of life.[30] However, the exact relationship between chronic pain and T2D remains unclear.

A potential relationship between chronic pain and T2D might include 2 aspects: the influence of T2D on nociception and the effect of chronic pain on the progression of T2D. As to the former, studies found that: 1) Impaired peripheral nerve insulin receptor (IR) signaling coincides with early thermal and tactile hypersensitivity in type 1 diabetic rats[24]; 2) insulin resistance, with or without compensatory hyperinsulinemia, is associated with altered nociception in T2D rats[25]; 3) in nondiabetic rats hyperinsulinemia per se tended to show thermal and mechanical hypoalgesia[25]; and 4) consequences of insulinemia or insulinemia itself play a role in the impairment of mechanical nociception.[21] Together, these results indicate that diabetes might change nociception threshold, but the direction of such changes (increase vs decrease) is unclear. Even less is known regarding the effect of chronic pain on the progression of T2D.

The genetically leptin-receptor deficient Zucker Diabetic Fatty (ZDF) rats develop obesity, insulin resistance, and T2D naturally. In this study, we used ZDF rats as a diabetic model and Zucker Lean (ZL) rats as controls to study a correlative relationship between the progression of T2D and changes in nociceptive threshold. We also examined whether there is a downregulated expression of IRs in the central nervous system in this process.

Methods

Diabetic Animal Model

ZDF (fa/fa; n = 35) rats and ZL (+/fa; n = 21) littermates 5 weeks old were purchased from Vital River Laboratories International Inc (Beijing, China). The number of rats was calculated using power analysis (.8), considering the data variation of postprocedure nociceptive behavior and of blood glucose level in ZDF rats. Littermates from the same or foster mother were housed in a specific pathogen-free condition, in a large plastic cage with wood chip bedding, distilled water, and standard rat diet pellets available ad libitum. Animals were housed under controlled temperature (21°C ± 2°C), relative humidity (50% ± 10%) and artificial light (12-hour light and dark cycle, lights on at 7 AM). Rats entered the experimental procedures (divided into 3 separate experiments, A, B, and C) at 6 weeks of age, as shown in Fig 1. We used only male ZDF and ZL littermates to avoid a possible confounding effect from sex and age differences on the levels of endogenous glucagon, insulin, and other possible hormones. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences. The care and use of animals conformed to applicable national and international guidelines. Principles of laboratory animal care were followed.

Preparation of Chronic Constriction Injury or Sham Animals

In this study chronic constriction injury (CCI) of a unilateral sciatic nerve was produced by placing loose ligations at the common sciatic nerve according to the method of Bennett and Xie.[5] Briefly, under 2% isoflurane anesthesia, the right side sciatic nerve was exposed in the mid thigh and 4 loose ligatures with 1.0- to 1.5-mm intervals were made around the nerve trunk using 4-0 chromic gut suture. The nerve trunk was then put back in its original position and the wound closed using sterilized wound clips. For sham rats we made the same surgical procedure except for the nerve ligation. No further analgesics were used after the CCI or sham procedure to avoid any unwanted effects.

Behavioral Testing

Experiment A was meant to compare the nociceptive threshold at baseline and after the CCI procedure

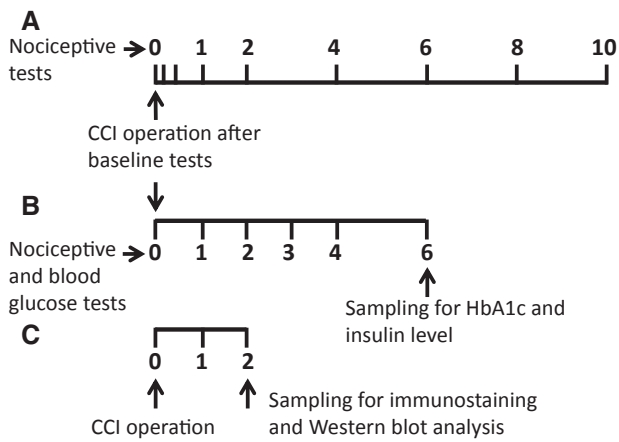

Figure 1. Experimental arrangement and time points in weeks. Showing the time points of events and the animal arrangement in 3 separate experimental processes: **(A)** nociceptive tests; **(B)** nociceptive and blood glucose tests; **(C)** sampling in CCI operated animals. Numbers and time points are in weeks.

between ZDF (n = 7) and ZL (n = 5) rats without use of the sham procedure for the purpose of conserving rats. A ZDF rat was excluded from further experiments because of postoperative paralysis and poor grooming.

The withdrawal threshold to thermal and mechanical stimulation was examined in each animal separately for the ipsilateral and contralateral hind paw before (baseline) and at various postprocedure time points up to 10 weeks. Animals were habituated to the test environment (behavior testing room) daily (a 60-minute session) for 2 days before baseline testing. All testing was conducted between 9:00 AM and 1 PM by an experimenter (C.S.) who was blinded to the group assignment.

To measure tactile sensitivity threshold, rats were placed into a plastic cage with a wire mesh bottom and examined by applying a von Frey filament to the plantar surface of each hind paw.[26,28] The von Frey filament set has a calibrated range of bending force (26, 15, 10, 8, 6, 4, 2, 1.4, 1, .6, .4, .16, and .007*g*). A single filament was applied to the plantar surface 5 times with an interstimulation interval of 5 seconds. A positive response was defined as at least 1 clear withdrawal response in the 5 applications. The filament was applied in an up-and-down fashion according to a negative or positive response.

Thermal hypersensitivity to radiant heat was determined according to a previously described method[12,28] using a 390 Analgesia Meter (IITC Inc, Woodland Hills, CA). Briefly, rats were placed individually into a plexiglass cubicle on a transparent glass surface. The light source from a projection bulb, located below the glass, was directed at the plantar surface of 1 hind paw. The foot withdrawal latency was defined as the time from the onset of radiant heat to withdrawal of the tested paw. The radiant heat source was adjusted to result in baseline latencies of approximately 12 seconds and a cutoff time at 20 seconds. Two trials with an intertrial interval of 5 minutes were made for each hind paw and scores from both trials were averaged to yield mean withdrawal latency for the hind paw.

Glucose Concentration Testing

In experiment B, rats (ZDF, n = 18; ZL, n = 10) were tested for random glucose concentration using an Ascensia Breeze Blood Glucose Monitoring System (Newbury, Berks, United Kingdom). The glucose concentration is used as a routine test to determine the progression of blood glucose metabolism dysfunction. Tail tip blood samples were taken between 9- and 10 AM while rats were restrained in a plastic cone. The blood glucose concentration test range was .6 to 33.3 mmol/L. Any concentration greater than the testing limit was recorded as 33.3 mmol/L for statistical purposes.

Plasma Glycohemoglobin Concentration

Hemoglobin A1c (HbA1c) is a minor hemoglobin component of erythrocytes and its sugar moiety is glucose covalently bound to the terminal amino acid of the β chain. Because glycohemoglobin concentration is not influenced by short-term blood glucose fluctuations over the preceding several weeks, the concentration of HbA1c is a more reliable index of the blood sugar average over a long period of time and is used to evaluate the development of a diabetic condition.

For analysis of plasma HbA1c concentration, blood samples were taken from left heart ventricles upon sacrifice, centrifuged for 10 minutes at 110*g*, 4°C to obtain plasma. All plasma sample–containing tubes were wrapped in foil and stored at −80°C until use. The concentration of plasma HbA1c was evaluated using enzyme-linked immunosorbent assay kits purchased from R&D Systems (Beijing, China) and analyzed by Huanya Biomedicine Technology Co LTD (Beijing, China). Results were read using a microplate reader (Multiskan MK3; Thermo Scientific, Beijing, China) at wavelengths of 450 nm.

Western Blot Analyses

In experiment C, ZDF (naive and CCI, n = 4 each) and ZL rats (naive and CCI, n = 2 each) were sacrificed 2 weeks after the procedure. Fresh tissues of spinal cord dorsal horn from the lumbar enlargement (left and right separately) and skeletal muscles were collected and saved at −80°C until use. The samples were homogenized in sodium dodecyl (lauryl) sulfate (SDS) buffer containing a mixture of proteinase inhibitors (Sigma, St. Louis, MO). Protein samples were separated on an SDS-polyacrylamide gel electrophoresis gel (4–15% gradient gel; Bio-Rad, Hercules, CA) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked with 3% milk and incubated overnight at 4°C with a primary antibody to IR (95 kD, rabbit polyclonal anti-IR β antibody [C18C4; ab69508], 1:5,000; Abcam, Cambridge, MA). After rinsing with phosphate-buffered saline 3 times for 10 minutes, the membranes were incubated at room temperature with horseradish peroxidase–conjugated secondary antibody (1:10,000; Abcam) for 1 hour. The blots were visualized in ECL solution (NEN, Boston, MA) for 1 minute and exposed on hyperfilms (Amersham Biosciences, Beijing, China) for 1 to 10 minutes. The membranes were then incubated in a stripping buffer (67.5 mm Tris, pH 6.8, 2% SDS, and 0.7% β-mercaptoethanol) for 30 minutes at 50°C and reprobed with a polyclonal rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:20,000; Beijing TDY Biotec, Beijing, China) as a loading control. The Western blot analysis was done in triplicate to produce a quantitative result. The density and size of the bands was measured with a computer-assisted imaging analysis system and normalized against loading controls.

Immunohistochemical Staining

Rats (ZDF, n = 2; ZL, n = 2) were anesthetized with pentobarbital (60 mg/kg, intraperitoneally) and transcardially perfused with 200 mL cold saline followed by 400 mL cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.35). Tissues of brain and spinal cord were dissected, postfixed for 2 hours, and kept in 30% sucrose in 0.1 M phosphate buffer at 4°C until they sank to the

bottom. Tissues were then cut using a cryostat (30 μm) and mounted onto microscope slides. Immunohistochemical staining was used to detect the qualitative expression of IR (rabbit polyclonal anti-IR β antibody [C18C4], 1:2,000; Abcam). For double immunolabeling of IR and neuronal nuclei (NeuN; mouse monoclonal anti-NeuN antibody, 1:1,000; Abcam) or IR and glial fibrillary acidic protein (GFAP; chicken polyclonal anti-GFAP antibody, 1:1,000; Abcam) primary antibodies were incubated together. Sections were blocked with 1% goat serum in 0.3% Triton X-100 for 1 hour at room temperature and incubated overnight at 4°C with the primary antibody. For controls, the primary antibody was omitted. The sections were then incubated for 1 hour at room temperature with corresponding fluorescein isothiocyanate- or cyanine dye 3-conjugated secondary antibody (1:200; Jackson ImmunoResearch, West Grove, PA). Slides were read using a LEXT OLS4000 3D Laser Measuring Microscope (Olympus, Center Valley, PA) and recorded using a digital camera.

Statistical Analysis

The raw data from blood glucose tests, HbA1c, and nociceptive sensitivity tests were analyzed using 1-way repeated measures analysis of variance across testing time points to detect overall differences among treatment groups (SigmaPlot version 11.0 for Windows; San Jose, CA). When significant main effects were observed, the Holm–Sidak tests were performed to determine sources of differences. Data from enzyme-linked immunosorbent assay and Western blot analyses were analyzed using the Student t-test to detect differences between treatment groups. The data are presented as mean ± standard error. Differences were considered to be statistically significant at the level of $\alpha = .05$.

To determine the association of nociceptive threshold to glucose concentration in ZDF rats, data were analyzed using SigmaPlot by comparing raw data at different time points (0, 2, and 6 weeks) in separate CCI and naive groups.

Results

Lower Nociceptive Threshold and Prolonged Recovery Period of Nociceptive Sensitivity in ZDF Rats

In naive rats, baseline nociceptive thresholds to thermal and mechanical stimulation were lower in ZDF rats than in ZL littermates at the age of 6 weeks. After the CCI procedure, ZDF and ZL rats developed thermal and tactile hypersensitivity. Although it took approximately 6 weeks after the procedure for the lower nociceptive thresholds to restore in ZL rats, it took approximately 8 weeks after the procedure for thermal hypersensitivity, and 10 weeks after the procedure for mechanical hypersensitivity to restore in ZDF rats (Fig 2).

Deteriorated Glucose Metabolism in ZDF Rats With CCI

As detected between 6 weeks (used as baseline) and 12 weeks of age, the blood glucose concentration in naive ZDF rats gradually increased, such that, by 10 weeks of age, the glucose concentration was significantly higher than baseline. In ZDF rats with CCI, beginning from the second postprocedure week (ie, 8 weeks of age) the blood glucose was already significantly higher. Beginning from the third postprocedure week, CCI rats showed a consistently higher blood glucose concentration than in naive ZDF rats (Fig 3A). Hyperglycemia related to nociceptive hypersensitivity was not present in ZL rats (Fig 3B). Although the sham-CCI rats showed a brief nociceptive sensitivity of 1 to 3 days, there was no observable blood glucose level change at any experimental time point (results not separately shown). This result indicates that a brief (acute) hypersensitivity might not induce a significant influence on the blood glucose level. At 12 weeks of age, the concentration of HbA1c in naive ZDF rats was higher than in naive ZL littermates. Although the concentration of HbA1c in ZL rats with CCI did not differ from that in naive ZL rats, it was significantly increased in ZDF rats with CCI compared with naive ZDF rats (Fig 4A).

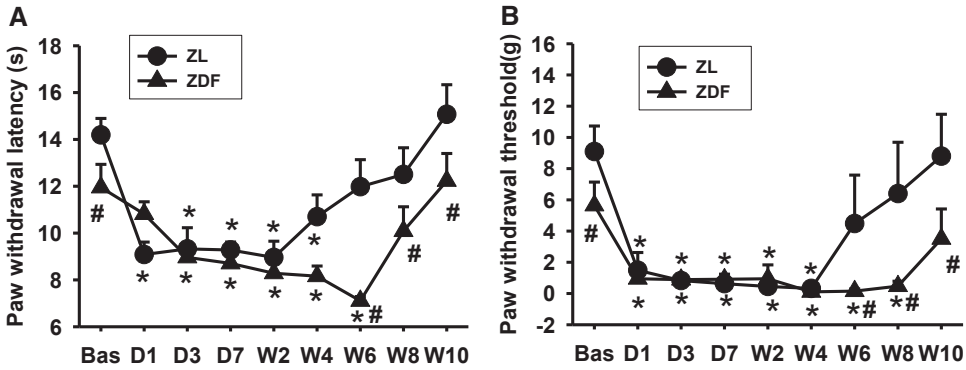

Figure 2. Differential nociceptive thresholds and nociceptive hypersensitivity recovery periods between ZDF and ZL rats. Naive ZDF rats showed a lower baseline nociceptive threshold to **(A)** thermal and **(B)** mechanical stimuli. There is also a prolonged recovery period for **(A)** thermal and **(B)** mechanical hypersensitivity after the CCI procedure in ZDF rats. *$P < .05$ compared with baseline (Bas) of the same group; #$P < .05$ compared with ZL at the same time point. Abbreviations: Bas, baseline data before the procedure; D, postprocedure time in days; W, postprocedure time in weeks.

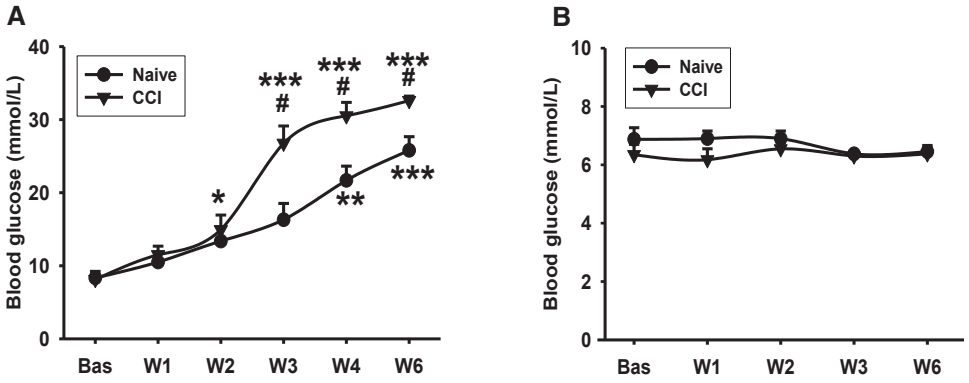

Figure 3. Influence of CCI on blood glucose concentration. There was an accelerated progression of hyperglycemia in **(A)** ZDF but not **(B)** ZL rats after the CCI procedure. *, **, ***, $P < .05, .01, .001$ compared with baseline of the same group, respectively; #$P < .05$ compared with the naive group at the same time point. Abbreviations: CCI, chronic sciatic nerve constriction injury group of rats; Bas, baseline data before the procedure; D, postprocedure time in days; W, postprocedure time in weeks.

Paradoxically, the insulin concentration at the time of sampling was higher in naive ZDF than in ZL rats. The CCI procedure in ZDF rats increased insulin to a significantly higher level in contrast to the change in ZL rats with CCI (Fig 4B). Combined with the hyperglycemia, these results indicate a deteriorated insulin resistance after the CCI procedure.

Correlation Between Nociceptive Threshold and Glucose Concentration in ZDF Rats

There was a correlation between nociceptive threshold and glucose concentration in CCI and naive ZDF rats (Fig 5). In ZDF rats with CCI, the correlation dots collectively shifted to the lower nociceptive area first and then to the higher glucose concentration area (Figs 5A and 5C). When data from all 3 time points (weeks 0, 2, and 6) were analyzed together, there was a strong negative correlation between glucose concentration and nociceptive threshold in CCI rats (for thermal stimulation, r = −.759; for mechanical stimulation, r = −.718), in contrast to a moderate negative correlation in naive ZDF rats (thermal, r = −.465; mechanical, r = −.209; Figs 5B and 5D, and Table 1).

Lower Expression of IR in the Skeletal Muscle and Spinal Cord of Naive ZDF Rats and Downregulated Expression of IR in CCI Rats

Western blot analysis showed that, although IR expression was comparable in liver and hypothalamus of naive ZL and ZDF littermates (data not shown), it was significantly lower in the skeletal muscles and the spinal cord dorsal horn of naive ZDF rats. This was further decreased in CCI rats 2 weeks after the procedure, particularly in ZDF rats (Fig 6). Additionally, immunofluorescence results showed that IR expression in the ipsilateral spinal cord dorsal horn was lower in ZL rats with CCI (Fig 7). Double-labeling immunofluorescence showed that IR expression in the brain was colocalized with the neuronal marker NeuN (Figs 8A–8H) but not the glial marker GFAP (Figs 8I–8L). Every IR-positive cerebral cell was also NeuN-immunoreactive but only a small of number NeuN-positive cells were IR-immunoreactive.

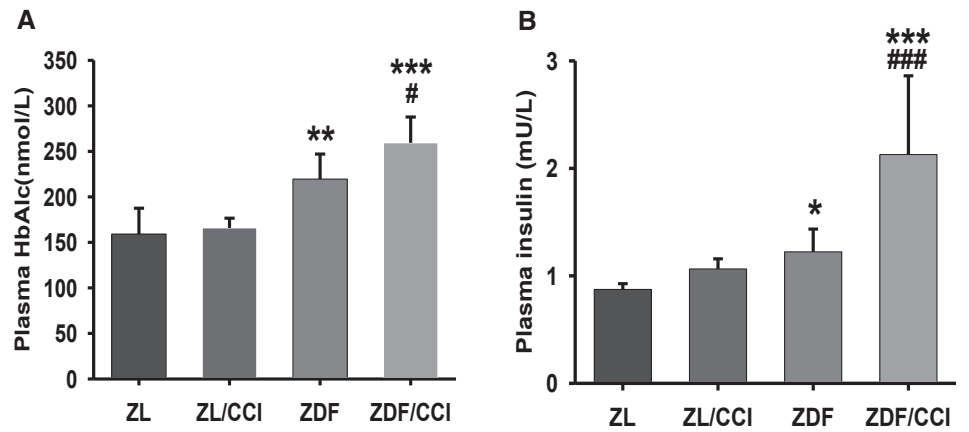

Figure 4. Differential influence of CCI on the concentration of HbA1c and insulin in ZDF and ZL rats. CCI induced higher **(A)** HbA1c and **(B)** hyperinsulinemia in ZDF but not ZL rats 6 weeks after the CCI procedure. *, **, ***, $P < .05, .01, .001$ compared with naive ZL, respectively; #, ###, $P < .05, .001$ compared with naive ZDF, respectively.

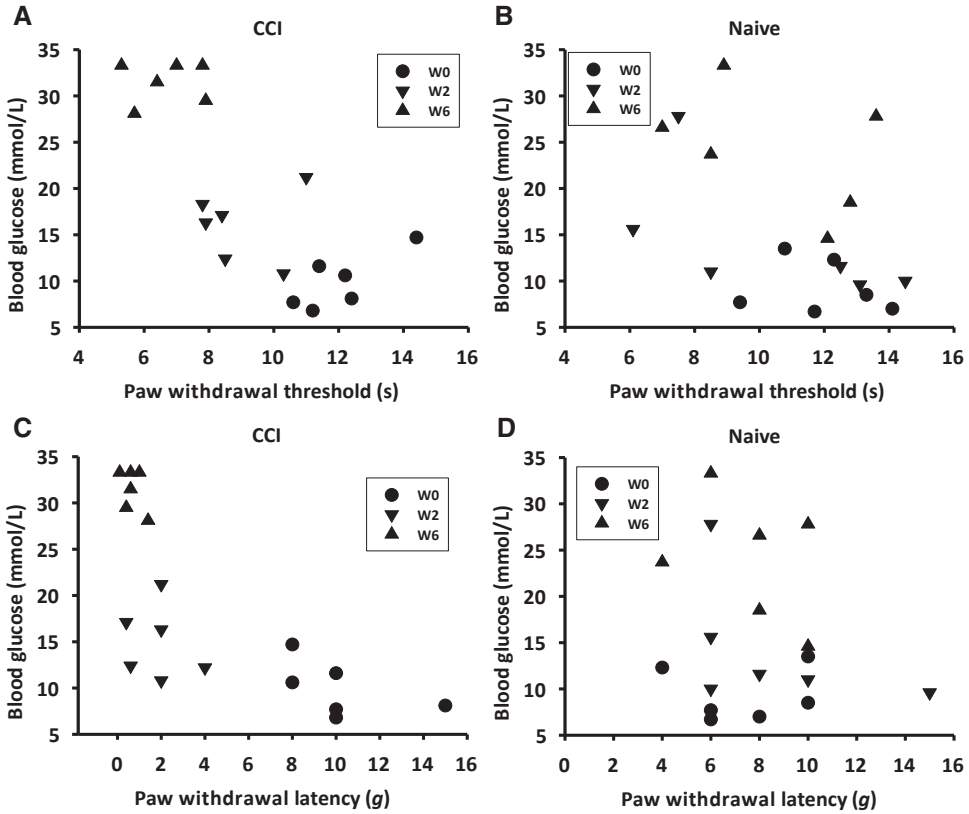

Figure 5. Association between nociceptive threshold and glucose concentration. **(A)** Thermal hyperalgesia threshold and **(C)** mechanical allodynia latency are negatively associated with blood glucose concentration. **(A)** and **(C)**There was a strong correlation in rats that received the CCI procedure and **(B)** and **(D)** a moderate correlation in naive ZDF rats. This suggests that CCI might deteriorate the glucose metabolism dysfunction (hyperglycemia) in ZDF rats. Abbreviations: W0, W2, W6, Week 0, 2, and 6, respectively.

However, a much higher number of NeuN-positive cells were also IR-positive in the spinal cord dorsal horn compared with in the brain.

Conclusions

In this study we found that: 1) ZDF rats have a lower nociceptive threshold, 2) ZDF rats with CCI showed a delayed restoration from nociceptive hypersensitivity, and 3) the presence of nociception in ZDF rats accelerated the progression of T2D.

Table 1. Correlations Between Glucose Concentration and Nociceptive Threshold in ZDF Rats

Variable	*CCI*	*Naive*
Estimated glucose level from thermal threshold	y = 46.087-2.918x R = −.759	y = 31.964-1.472x R = −.465
Estimated glucose level from mechanical threshold	y = 25.315-1.54x R = −.718	y = 20.947-0.647x R = −.209
Estimated thermal threshold from glucose level	y = 13.009-0.197x R = −.759	y = 13.252-0.147x R = −.465
Estimated mechanical threshold from glucose level	y = 10.517-0.334x R = −.718	y = 8.907-0.068x R = −.209

ZDF rats have a missense mutation in the leptin receptor gene and are widely used for diabetes and obesity studies. Leptin is an adipokine and hormone that plays a key role in regulating energy intake and expenditure, including appetite and hunger, metabolism, and behavior.[6] Leptin functions by binding to the leptin receptor. Without a functional leptin signaling system, the leptin receptor-deficient db/db mice develop T2D-like conditions, obesity, and hypertension. Additionally, db/db rodents exhibited features that could be found in humans with diabetic autonomic neuropathy and thus could serve as a preclinical model for this condition.[10] The CCI model in ZDF rats was used in this study to mimic diabetic neuropathy, because it provides conditions of obesity, a high level of blood glucose, chronic thermal and tactile hypersensitivity, and a mechanism of partial vessel interruption (arteriosclerosis in the arteriole) in the nerve trunk. Although ZDF rats naturally develop obesity, insulin resistance, and T2D, our results show that chronic pain accelerates the progression of these conditions.

It has been reported that at 8 weeks of age, ZDF rats develop T2D with insulin resistance and decreased β-cell function.[1] In the current study, ZDF rats at 6 weeks of age showed signs of hyperglycemia with lower nociceptive thresholds to thermal and mechanical stimuli, compared with naive ZL rats. The leptin signaling system has been proven to be involved in nociceptive

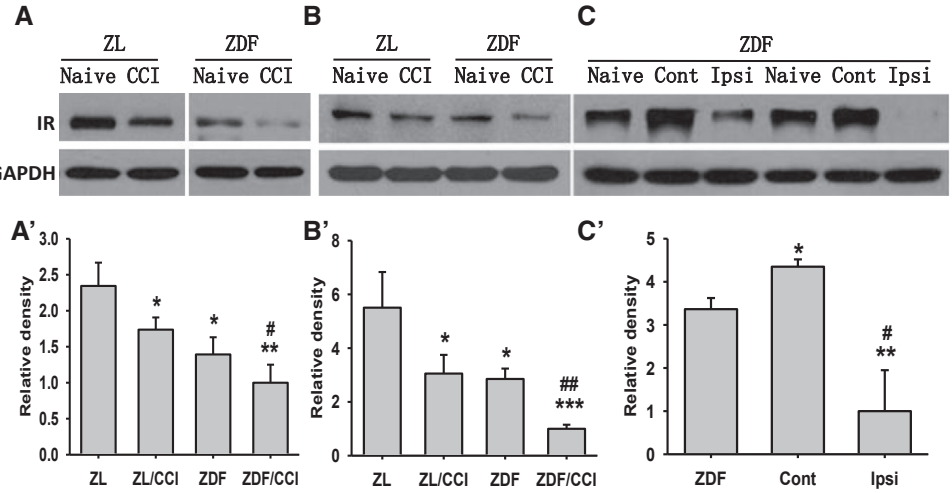

Figure 6. Expression of IR in **(A)** spinal cord dorsal horn and **(B)** and **(C)** skeletal muscles of naive and CCI rats. Western blots show a lower expression IR in **(A)** spinal cord and **(B)** skeletal muscles of naive ZDF rats as compared with that in naive ZL rats. Two weeks after the CCI procedure the expression of IR was further downregulated in ZL and ZDF rats, with a stronger effect in ZDF rats **(B)**. *, **, ***, $P < .05, .01, .001$, respectively, compared with naive ZL rats. #, ##, $P < .05, .01$, respectively, compared with naive ZDF rats. Compared within ZDF rats alone, the expression of IR in the skeletal muscle directly innervated by CCI procedure nerve was significantly downregulated and it was somewhat upregulated in the contralateral skeletal muscle **(C)**. **(A')**, **(B')**, and **(C')** show relative density (in pixels) of IR blots in panels **(A)**, **(B)**, and **(C)**, respectively. *, **, $P < .05, .01$, respectively, compared with naive ZDF rats. #$P < .05$, compared with contralateral. Abbreviations: Cont, skeletal muscle innervated by contralateral sciatic nerve; Ipsi, skeletal muscle directly innervated by CCI procedure sciatic nerve.

sensitivity.[27] The different nociceptive sensitivity between ZDF and ZL rats of 6 weeks of age might be because of the leptin receptor expression deficit in ZDF rats. Notably, after another 6 weeks (12 weeks of age) in a neuropathic pain condition, ZDF rats showed hyperinsulinemia and a higher HbA1c level, and naturally at 12 weeks of age, the insulin concentration in naive ZDF rats was not significantly higher than that in ZL rats. These results indicate that: 1) lower nociceptive thresholds and hyperglycemia developed early, 2) they might develop simultaneously, 3) CCI provides additive hyperalgesia to diabetes and the eventual nociceptive recovery does not include restoration to nondiabetic values; and 4) a condition of chronic nociception hypersensitive exacerbates insulin resistance in ZDF rats. The correlation between nociceptive threshold and blood glucose concentration supports these viewpoints. Recovery of hyperalgesia in nerve injury models has been attributed to regeneration and reinnervation.[9] The delayed nociceptive hypersensitivity recovery seen in the diabetic rats with CCI could arise from the delayed nerve regeneration that is commonly reported in diabetic rodents.[13] However, our results also suggest that the downregulated expression of IRs might be part of a shared mechanism between insulin resistance and nociceptive hypersensitivity.

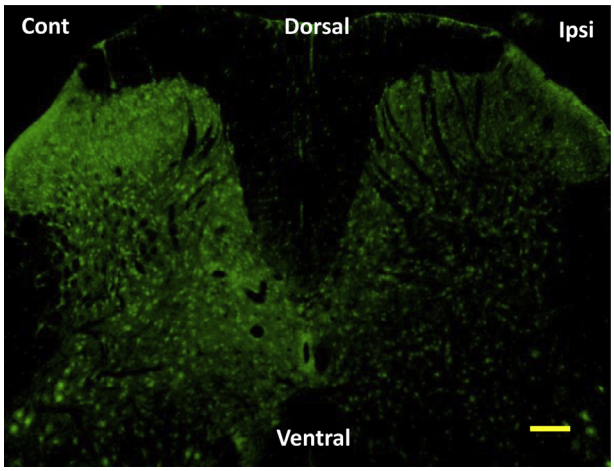

Figure 7. Expression of IR-positive cells in the spinal cord of a ZL rat 2 weeks after the CCI procedure. There is a downregulated expression of IR-immunopositive cells (green) in the spinal cord ipsilateral to the CCI procedure. Bar, 250 μm. Abbreviations: Cont, control lateral to the CCI procedure; Ipsi, ipsilateral to the CCI procedure; Dorsal, dorsal part of spinal cord; Ventral, ventral part of spinal cord.

Biological actions of insulin are initiated by binding to its cell surface receptor, which results in autophosphorylation of the receptor, activation of its intrinsic tyrosine kinase activity,[20,22] and finally, activation of intracellular pathways.[29] Insulin receptors are also widely expressed in peripheral neurons in the cell body and in axons.[3,4,18] Neurons indeed develop insulin resistance after hyperinsulinemia in a manner similar to that in metabolic tissues.[17] In a previous study, after diabetic neuropathy was induced in a type 1 diabetes rat model, treatment with low-dose insulin normalized behavioral scores in 5 weeks, although severe hyperglycemia remained, indicating that impaired insulin signaling does play a role in the pathophysiology of painful diabetic neuropathy.[14]

Acute pain has been reported to reduce insulin sensitivity in patients due to a decreased glucose uptake in the body.[11] In this study, CCI resulted in an acute condition of hypersensitivity and transition to a chronic condition at 8 weeks. Although the behavior eventually recovered with time, the T2D persisted. Therefore, although the

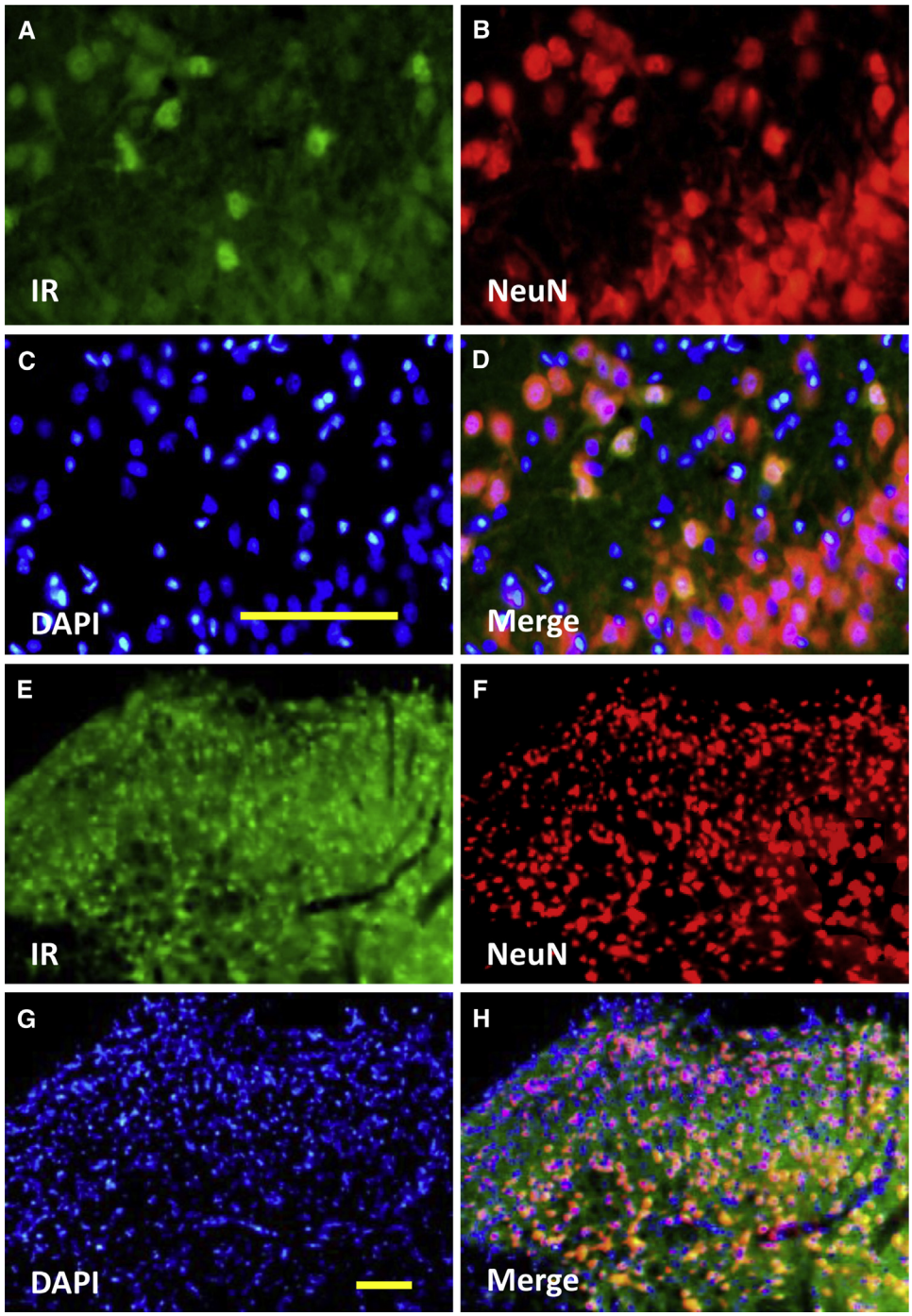

Figure 8. Colocalization of IR-immunoreactivity with NeuN but not GFAP in the brain and spinal cord dorsal horn of ZL rats. Although IR-positive cells (green) were all NeuN-reactive in **(A-D)** brain and **(E-H)** spinal cord dorsal horn, a much higher ratio of the NeuN-positive cells were IR-positive in the spinal cord dorsal horn compared with in the brain. There was no colocalization between IR (green) and GFAP **(I-L)**. Bar, 100 μm. Abbreviation: DAPI, 4′,6-diamidino-2-phenylindole.

change in sensitivity reduced insulin sensitivity, there might be other mechanisms for the accelerated T2D progression induced by CCI. In this study, we examined the expression of IRs in the spinal cord and skeletal muscles in naive ZDF and ZL rats, as well as in CCI rats 2 weeks after the procedure. We found that the expression level of IRs was lower in tissues taken from naive ZDF rats and was even lower in ZDF and ZL rats with CCI. Our data suggest that the downregulation of IRs secondary to CCI might occur in multiple loci in the body, including the central nervous system, liver, skeletal muscle, and adipose and other metabolic tissues, which might serve as a possible mechanism underlying a reciprocal relationship between chronic pain and the progression of T2D.

Because IR is necessary for the body cells to use glucose, the IR signaling system in the skeletal muscle might be involved in the motor function of the muscle. However, the low expression of IR in skeletal muscles

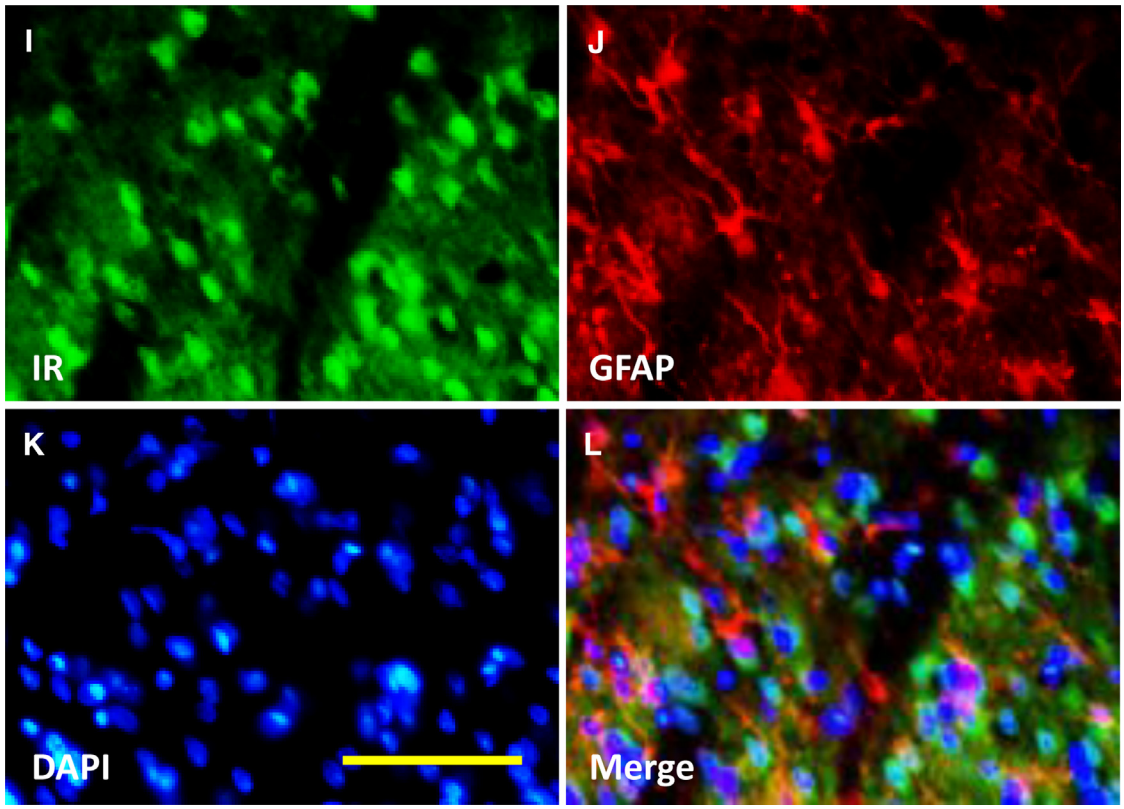

Figure 8. *(continued)*

most likely would delay the behavioral response instead of shorten the response time. Thus the behavioral sensitivity to thermal and mechanical stimuli in ZDF rats, especially after the CCI procedure, might not be the results of motor function failure.

Previous studies have shown that: 1) obesity is the main cause of T2D[23] and over a third of American adults are considered obese,[8] 2) the prevalence of chronic pain varied from 10.1% to 55.2% of the population around the world,[7,16] 3) as of 2013, it was estimated that there were 382 million people with T2D worldwide, making up approximately 90% of diabetes cases and equivalent to approximately 8.3% of the world's adult population,[15] and 4) in the United States alone, 11.3% of adults aged 20 years and older have T2D and this increases to 25.9% in adults aged 65 years and older.[2,19]

Our results showed that T2D exacerbates chronic pain and chronic neuropathic pain accelerates the progression of T2D in ZDF rats. Although the exact cellular mechanism of this relationship remains to be elucidated, our data suggest that adequate treatment of chronic pain in patients who are genetically and environmentally susceptible to obesity and diabetes might be a key step to break a vicious cycle of T2D and pain progression.

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