Table 1 Clinical characteristics of participants with IFG and/or IGT at baseline according to change in glucose tolerance during the observation period

Characteristics	'Recovered'	'Persistent'	'DM'
No. of participants	39	53	36
Age (years)	47.7 ± 6.4	49.6 ± 5.6	50.5 ± 5.8
Height (cm)	167.6 ± 5.6	168.4 ± 4.6	168.1 ± 5.8
Weight (kg)	69.7 ± 7.4	70.1 ± 8.7	70.4 ± 9.2
Body fat (%)	25.4 ± 4.4	25.5 ± 5.6	25.2 ± 6.2
Body mass index (kg/m^2)	24.8 ± 2.3	24.5 ± 5.6	24.9 ± 3.3
Systolic blood pressure (mmHg)	129 ± 13	127 ± 14	126 ± 12.0
Diastolic blood pressure (mmHg)	81 ± 11	83 ± 10	81 ± 10
AST (IU/l)	26 ± 6.5	30 ± 11.9	32 ± 13.2
ALT (IU/l)	29 ± 12.1	38 ± 24.0	38 ± 24.7
LDH (IU/l)	315 ± 62.8	326 ± 56.0	344 ± 75.0
γ-GTP (IU/l)	82 ± 54.5	80 ± 66.5	85 ± 54.4
ALP (IU/l)	195 ± 52.3	203 ± 50.8	218 ± 53.9‡
Total protein (g/l)	72 ± 3.8	72 ± 3.7	72 ± 3.9
Creatinine (μmol/l)	84 ± 12.4	84 ± 13.3	86 ± 10.6
Total cholesterol (mmol/l)	5.5 ± 0.80	5.6 ± 0.95	5.3 ± 0.95
Triglycerides (mmol/l)	1.9 ± 1.12	1.9 ± 1.05	1.6 ± 0.90
HDL-C (mmol/l)	1.5 ± 0.32	1.4 ± 0.41	1.4 ± 0.22
LDL-C (mmol/l)	3.3 ± 0.61	3.3 ± 0.77	3.2 ± 0.84
Uric acid (mg/l)	61.7 ± 13.4	60.0 ± 11.3	59.0 ± 11.2
S-Amylase (IU/l)	88.5 ± 20.4	87.9 ± 20.6	101.3 ± 35.9
ESR (mm/h)	5.9 ± 5.8	6.8 ± 7.5	8.5 ± 7.8*‡
WBC (/μl)	6481 ± 1846	6222 ± 1873	6096 ± 1476
RBC (×10^4/μl)	490.6 ± 35.7	486.8 ± 35.0	479.8 ± 40.7
Haemoglobin (g/dl)	15.4 ± 1.0	15.5 ± 0.9	15.3 ± 1.2
Haematocrit (%)	48.3 ± 2.7	48.6 ± 2.6	47.6 ± 3.7
FPG (mmol/l)	5.5 ± 0.5	5.7 ± 0.6	6.1 ± 0.6†§
1 h-glucose (mmol/l)	10.6 ± 2.2	11.1 ± 2.0	11.3 ± 2.1
2 h-glucose (mmol/l)	8.4 ± 0.8	8.7 ± 0.8	8.8 ± 1.3
Urine protein (+/–)	2/37	2/51	2/34
Fatty liver (+/–)	10/29	14/39	14/21
Night duty (+/–)	4/30	4/42	8/26
Blue-collar worker (+/–)	28/11*	48/5	27/9*
Administrative position (+/–)	0/39	0/53	1/35
Business bachelor (+/–)	3/36	7/46	10/26‡
Stress in daily life (+/–)	4/35	8/45	6/30
Satisfaction with lifestyle	5.13 ± 3.11	5.44 ± 2.38	6.49 ± 3.78
Egogram (CP > NP)	11/28	7/46	8/28
Egogram (AC > FC)	12/27	13/40	9/27
Fatigue (grade 1–4)	0.69 ± 1.03	1.00 ± 1.22	1.00 ± 1.22
Alcohol drinking (+/–)	36/3	41/12	33/3
Current smoking (+/–)	12/27	25/28	17/19

*$P < 0.05$; †$P < 0.01$ ('Persistent' vs. 'Recovered' or 'DM'); ‡$P < 0.05$; §$P < 0.01$ ('Recovered' vs. 'DM').
AC, adapted child; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; CP, critical parent; DM, diabetes mellitus; ESR, erythrocyte sedimentation rate; FC, free child; FPG, fasting plasma glucose; γ-GTP, gamma-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LDH, lactic dehydrogenase; LDL-C, low-density lipoprotein cholesterol; NP, nurturing parent; RBC, red blood cell; WBC, white blood cell. ['Recovered', participants with recovery of NGT from IFG and/or IGT at baseline during the observation period; 'Persistent', participants with persistent IFG and/or IGT; 'DM', participants with progression to DM from IFG and/or IGT.]

DM from IFG and/or IGT, as well as factors associated with the recovery of NGT from IFG and/or IGT, by adjusting for age, BMI, systolic blood pressure, ALT, LDH, γ-GTP, ALP, TP, Cr, TG, HDL-C, LDL-C, UA, S-amylase, ESR, WBC, Hb, FPG, urinary protein, night duty, blue-collar job, administrative position, business bachelor, stress in daily life, satisfaction with lifestyle, Egogram, fatigue, alcohol drinking and smoking status. As shown in Table 2, night duty ($P < 0.01$), higher FPG within the range 6.1–6.9 mmol/l ($P < 0.05$), stress in daily life ($P < 0.05$) and having an administrative position ($P < 0.05$) were significant independent risk factors for the development of DM from IFG and/or IGT. Being a business bachelor did not reach statistical significance. In contrast, lower FPG, within the range 6.1–6.9 mmol/l ($P < 0.05$), having a white-collar job ($P < 0.05$), being a non-smoker ($P < 0.05$) and having lower serum ALT levels ($P < 0.05$) were significant independent factors for the recovery of NGT from IFG and/or IGT (Table 3). Lower systolic blood pressure did not reach significance.

Table 2 Multiple regression analysis of risk factors for the progression to Type 2 diabetes from IFG and/or IGT

	P	HR	95% CI
Night duty	0.002*	5.48	1.82–16.49
FPG	0.031*	1.05	1.01–1.10
Stress in daily life	0.037*	3.81	1.09–13.35
Administrative position	0.045*	12.70	1.06–150.83
Business bachelor	0.064	2.68	0.95–7.57

*$P < 0.05$.
Model: adjusted for age, body mass index (BMI), systolic blood pressure, alanine aminotransferase (ALT), lactic dehydrogenase (LDH), gamma-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), total protein, creatinine, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid, S-amylase, erythrocyte sedimentation rate (ESR), white blood cell (WBC), haemoglobin, FPG, urinary protein, night duty, blue-collar job, administrative position, business bachelor, stress in daily life, satisfaction with lifestyle, Egogram (CP > NP), Egogram (AC > FC), fatigue, alcohol drinking and current smoking.
AC, adapted child; CI, confidence interval; CP, critical parent; FC, free child; FPG, fasting plasma glucose; HR, hazard ratio; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NP, nurturing parent.

Table 3 Multiple regression analysis of beneficial factors for the recovery of NGT from IFG and/or IGT

	P	HR	95% CI
FPG	0.017*	0.94	0.894–0.989
Blue-collar worker	0.033*	0.34	0.127–0.917
Smoker	0.040*	0.31	0.098–0.948
ALT	0.042*	0.97	0.947–0.999
Systolic blood pressure	0.085	0.86	0.724–1.021

*$P < 0.05$.
Model: adjusted for age, body mass index (BMI), systolic blood pressure, alanine aminotransferase (ALT), lactic dehydrogenase (LDH), gamma-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), total protein, creatinine, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid, S-amylase, erythrocyte sedimentation rate (ESR), white blood cell (WBC), haemoglobin, FPG, urinary protein, night duty, blue-collar job, administrative position, business bachelor, stress in daily life, satisfaction with lifestyle, Egogram (CP > NP), Egogram (AC > FC), fatigue, alcohol drinking and current smoking.
AC, adapted child; CI, confidence interval; CP, critical parent; FC, free child; FPG, fasting plasma glucose; HR, hazard ratio; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NP, nurturing parent.

Discussion

To comprehensively determine factors affecting the progression of Type 2 diabetes and the recovery of NGT from IFG and/or IGT, we conducted a study in which we prospectively investigated Japanese workers with IFG and/or IGT, who are at high risk of developing diabetes [14,15]. We found that, in addition to FPG levels, social and psychological factors such as status at work (night duty), stress in daily life and social position (administrative position) are also independent risk factors for the progression to DM from IFG and/or IGT, while being a white-collar worker and a non-smoker are factors associated with the recovery of NGT.

In our study, the rate of development of overt diabetes from IFG and/or IGT was equal to or relatively low (28.1%) and the rate (30.5%) of reversal of NGT was relatively high, compared with other reports [14–17]. There are several possible reasons for these differences: (i) participants were educated about dietary and exercise therapy once or twice a year by dieticians and medical doctors; (ii) participants were non-obese (mean BMI less than 25.0 kg/m^2); and (iii) the DM group gained only a small amount of weight (less than 1 kg over 3.2 years), whereas the recovered group lost a small amount of weight, approximately 0.1 kg (data not shown). These factors might reduce the development of diabetes.

Furthermore, known risk factors such as obesity and elevated liver enzyme levels [1–5] were not found to be risk factors for the development of diabetes from IFG and/or IGT in our study, probably because the majority of participants analysed were not obese and they were already at the highest risk; i.e. they had IFG and/or IGT. Among participants with IFG and/or IGT at baseline, those with higher FPG levels (6.1–6.9 mmol/l) might have greater impairment of insulin secretion than those with lower FPG levels, which were associated with the recovery of NGT from IFG and/or IGT.

The social and psychological factors, such as night duty, stress in daily life and administrative position, resulted in the progression from IFG and/or IGT to overt diabetes, possibly as a result of increasing insulin resistance. In our study, night duty was the highest risk factor for progression to diabetes. Subjects who are on night duty experience sleep disorders, which are known to affect the sympathetic nervous system and to be associated with impaired glucose tolerance [24]. In addition, short sleep duration is a risk factor for developing diabetes, independent of confounding factors [25]. Furthermore, stress in daily life and status at work (administrative position) were risk factors for the development of diabetes. Persons in administrative positions often experience both physical and mental stress [26]. In our case, because they were middle managers, they may have experienced strong job strain and stress as a result of high job demands combined with low job decision latitude and effort–reward imbalance, factors which are associated with Type 2 diabetes [7–11] and cardiovascular diseases [27–29].

It has been proposed that stress activates the hypothalamo–pituitary–adrenal axis and the central sympathetic system and leads to the development of endocrine perturbation and obesity, which increases insulin resistance, causing Type 2 diabetes [30,31]. In addition, increased levels of stress hormones such as catecholamines and glucocorticoids may impair insulin

secretion [32]. Furthermore, stress may induce pro-inflammatory cytokines [33] and DNA damage [34], factors which are related to Type 2 diabetes.

Interestingly, approximately one-third (30.5%) of participants with IFG and/or IGT at baseline returned to NGT. A multivariate analysis indicated that baseline factors related to improvement of glucose intolerance also included social factors (white-collar worker) and lifestyle (non-smoking), as well as lower levels of FPG (6.1–6.9 mmol/l) and serum ALT levels. Smoking is a risk factor for IGT and Type 2 diabetes [35,36], while serum ALT level is a risk factor for Type 2 diabetes [3,4]. Therefore, non-smoking and low serum ALT levels may be associated with a return to NGT from IFG and/or IGT.

Although this is one of the only studies to prospectively examine relationships between psychosocial factors and risk of diabetes, further study is necessary. One limitation of this study is that many variables were examined with only a small sample size.

In summary, the results of the present study indicate that, in addition to glucose levels, social and psychological factors also affect progression to Type 2 diabetes or recovery of NGT from IFG and/or IGT in Japanese workers and social and psychological interventions may need to be considered to prevent the development of Type 2 diabetes in those at high risk.

Competing interests

Nothing to declare.

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糖尿病性神経障害
—成因・診断・治療の新展開

S1-1. 糖尿病性神経障害における インスリン分泌・作用異常の関与

杉本 一博* 保嶋 実* 八木橋操六**

〔糖尿病合併症 22 (1) : 34～39, 2008〕

はじめに

元より糖尿病はインスリンの分泌，作用，あるいはその両方の異常によって引き起こされる疾患である。現行の臨床検査の指標の中で，このインスリンの分泌・作用異常をもっとも鋭敏に反映し得るのが糖代謝異常の慢性高血糖と言うことになる。しかしながら，この慢性高血糖に対する治療介入が，必ずしもその原因となるインスリン分泌・作用異常の是正に繋がっていない場合もある。インスリン分泌・作用異常の是正を伴わない慢性高血糖のみの改善は，場合によって糖尿病合併症を悪化させ得る。この1例として，急激な高血糖の改善によって誘導される急性有痛性神経障害(治療後神経障害)が考えられる。

近年，糖尿病性神経障害に特徴的な小径感覚神経障害が，前糖尿病状態である耐糖能障害 (IGT) においても高頻度に認められることが報告されている[1]。これらのIGT患者においては，慢性高血糖は糖尿病域にまで至らなくても，しばしばインスリンの分泌・作用異常，特に後者の主因であるインスリン標的臓器におけるインスリン感受性の低下は糖尿病患者のそれに十分匹敵している。末梢神経，特に小径感覚神経細胞には豊富なインスリン受容体の発現が認められる[2]。したがって，仮に慢性高血糖が軽微あるいは存在しなくても，背景に認められるインスリン分泌・作用異常が相当程度に達した場合，神経障害，特に小径感覚神経障害の発症に関与し得る可能性がある。

現在まで糖尿病性神経障害の基礎的研究に汎用されているストレプトゾトシン誘発糖尿病 (STZ-D) ラットモデでは，糖尿病発症後直ちに痛覚異常を含む種々の急性神経機能異常を認めるが，これらの異常はその後の長期観察においても進行することなく持続するのが特徴である。このSTZ-Dラットに見られる急性非進行性神経機能異常を慢性高血糖の結果とみなし，糖尿病患者に罹病期間依存性に生じる神経障害と一律に扱うことは妥当でない可能性がある。筆者は，このラットに認められる急性インスリン欠乏そのものが，末梢神経のインスリンシグナル異常を介して神経機能異常を引き起こしていると推定している。

本稿では，以上の3つの作業仮説について，筆者らの研究から得られた成績に文献的考察を加えて報告する。

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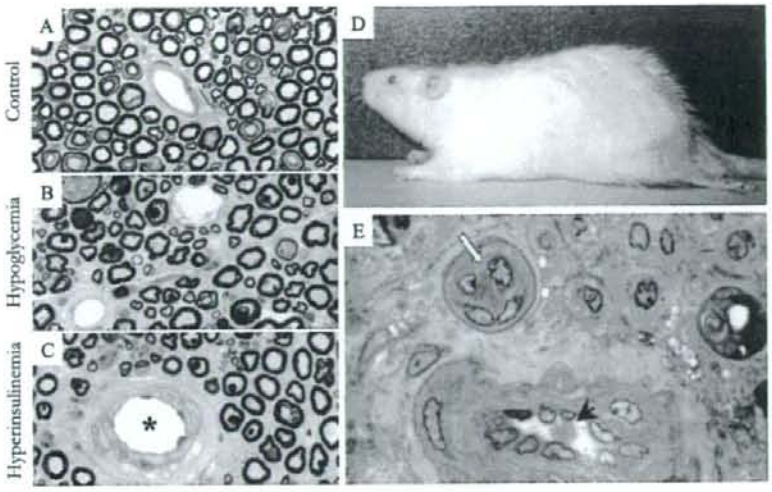

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Fig. 1 Representative photographs of transverse semithin sections of the sciatic nerve as well as a photograph of an insulinoma-bearing rat developing paresis of the hind limbs. In a control rat (A), there are only scattered myelinated fibers with vacuolar changes of myelin sheath. In an insulinoma-bearing rat showing significant hypoglycemia (B), there are a number of myelinated fibers undergoing axonal degeneration and axons surrounded by vacuolar or delaminated myelin sheath. A section from an insulinoma-bearing rat with significant hyperinsulinemia (C) contains an increased number of small-sized microvessels with plumped endothelial cells; a microvessel shows conspicuous microangiopathic changes such as vascular wall thickening and endothelial hyperplasia (asterisk). An insulinoma-bearing rat (D) was unable to stand on limbs and dragged hind limbs during movements. The sciatic endoneurial microvessels (E) from the paralytic animal with insulinoma show narrowing or occlusion (open arrow) of vascular lumina by swollen endothelial cells and/or thrombotic materials (arrow).

治療後神経障害とインスリノーマラットモデルの神経障害

1933年，Caravati[3]はインスリン治療に引き続いて生じる有痛性神経障害をインスリン神経炎（insulin neuritis）と名付け，その1症例を報告した。その後も同様な臨床経過を示す症例は多数報告されているが，このCaravatiが見出した患者が，現在我々が「治療後神経障害」と診断している病態を呈した最初の報告例とされている。この70年以上前より認知されている神経障害の正確な発症機序は未だ十分解明されていないが，Kiharaら[4]は，正常ラットを用いた実験を行い，血糖値とは無関係にインスリンそのものが神経周膜外動静脈シャント血流量を増加させ，神経内低酸素を誘導して末梢神経の機能異常を引き起こす可能性を示している。また，Tesfayeら[5]は，治療後神経障害（彼らは“急速血糖コントロールの急性有痛性神経障害”と呼んでいる）患者の露出腓腹神経を生体内蛍光血管造影法で観察することで，増殖性網膜症で認められる血管病変に類似した動静脈シャント，新生血管，血管透過性の亢進や拡張・蛇行した静脈を含む変化を神経周膜外血管に見出している。これらの血管病変は，治

療後神経障害における神経内虚血の存在を示唆している。筆者らは，長期インスリノーマラットモデルを用いて，慢性原発性高インスリン血症が，高度な神経内細小血管障害と神経変性・脱落を引き起こす可能性を示している[6,7]（Fig. 1）。特に，明らかな低血糖を示さなかった高インスリン血症ラットに，神経内細小血管障害が高度であったことから（Fig. 1-C），高インスリン血症そのものが末梢神経内血管病変の形成に寄与する可能性が示唆される。

一方で，インスリンには神経成長因子としての作用があり，インスリン投与によるインスリン欠乏の改善が，痛みの伝播に関与する小径線維の軸索発芽を促し，この再生神経からの異所性の神経活動電位の発生を一時的に増加させて急性有痛性神経障害を引き起こしている可能性も推定される。この仮説を実際の糖尿病患者で実験的に証明することは困難であるが，適切なインスリン治療を継続することで，やがて再生神経からの異所性神経活動電位の発生は減少し，有痛性神経障害の鎮静化に繋がる可能性がある。しかしながら，仮に患者がこのような良好な経過を辿ったとしても，過剰インスリンがもたらし得る末梢神経への影響に配慮した治療法の選択が求められると考える。

肥満関連インスリン抵抗性 Zucker および2型糖尿病 ZDF ラットモデルの痛覚反応の経時変化

インスリン抵抗性 IGT から2型糖尿病への耐糖能の悪化に伴って，小径感覚神経障害にいかなる変化が生じ，インスリン抵抗性に随伴するどのような代謝異常と関連を示しているのかはよく解っていない。筆者らは，肥満関連インスリン抵抗性およびインスリン抵抗性2型糖尿病ラットモデルを用いて，小径感覚神経機能の指標である温痛覚反応の経時変化を解析し，認められた変化とこれらのモデルのインスリン代謝異常との関連性を検索した。

肥満関連インスリン抵抗性動物モデルとして，雄性肥満 Zucker（OZ）ラットを用い，インスリン抵抗性2型糖尿病動物モデルとして，雄性 Zucker Diabetic Fatty（ZDF）ラットを用いた。対照として，それぞれ週齢および性を合わせたやせ形正常ラットを用いた。8週から36週齢まで経時的にラットの体重，血糖値および尾の温痛覚反応潜時（TFL, tail flick latency）を測定した。また，8–10，24および38–39週齢目に，各群の一部のラットについて血清インスリン値を測定した。

OZ ラットは8週齢より持続性の体重増加と10週齢より軽度の高血糖（150～250 mg/dl）を示した。この肥満モデルの TFL は8週齢で有意な短縮を示したが，32週齢以降では逆に有意に延長していた（Fig. 2-A）。血清インスリン値は，8および24週齢では有意に上昇していたが，38週齢ではやせ形正常ラットと同等であった（Fig. 2-B）。ZDF ラットは10週齢以降での重度の高血糖（400 mg/dl～）と8から14週齢までで有意な体重増加を示した。この2型糖尿病モデルの TFL は8週齢で有意に短縮していたが，16週齢以降では逆に有意に延長していた（Fig. 2-C）。血清インスリン値は10週齢で有意に上昇していたが，遅くとも24週齢にはやせ形正常ラットと同等であった（Fig. 2-D）。

インスリン抵抗性 OZ および ZDF ラットでは，持続性の高血糖を認める以前に TFL の短縮によって示される温痛覚過敏が出現おり，これらのラットにおける温痛覚過敏は，代償性高インスリン血症を伴うインスリン抵抗性の初期徴候の1つである可能性が示唆された。また，これらのラットでは，TFL の延長によって示される温痛覚鈍麻が，この高インスリン血症の消失時期と一致して認められた。以上の結果は，インスリン抵抗性ラットモデルに認められる小径感覚神経障害が，血糖値とは独立してインスリン代謝異常と関連性を示す可能性を示唆している。

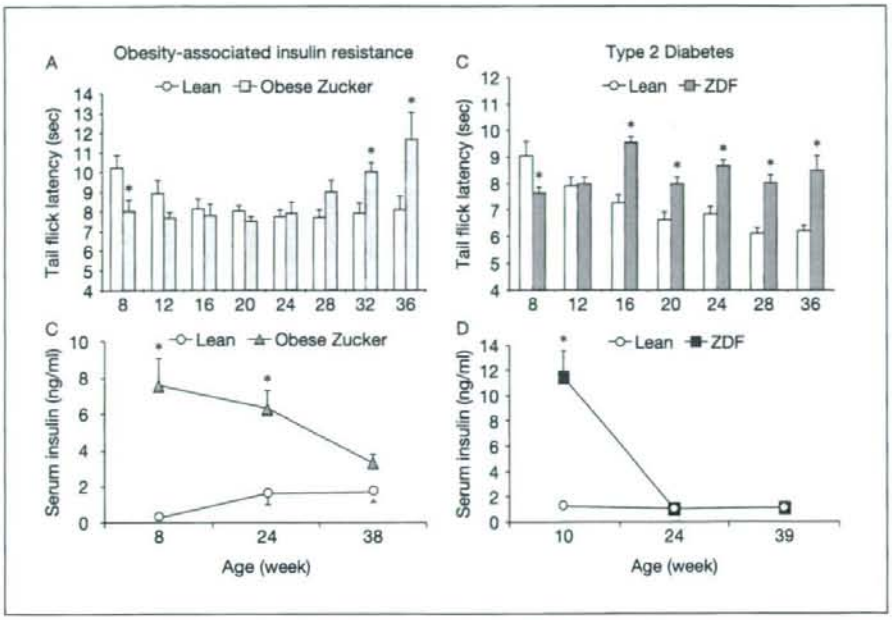

Fig. 2 Tail flick latency (A, C) and serum insulin levels (B, D) as a function of age in the insulin resistance (A, B) and the type 2 diabetes rat models (C, D). $*p < 0.05$ vs the age-matched control group.

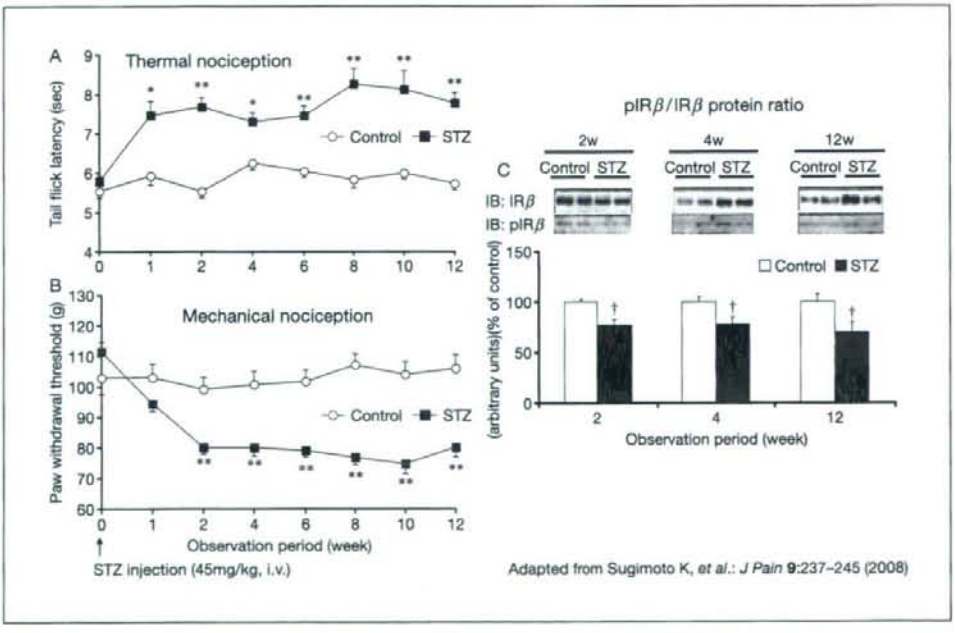

Fig. 3 Time course of changes in thermal (A) and mechanical (B) nociceptive responses as well as in peripheral nerve expression of tyrosine phosphorylated (Try1146) insulin receptor (pIR) relative to that of total insulin receptor (IR) β subunit in STZ-diabetic rats. $^{\dagger}p < 0.05$, $*p < 0.005$, $**p < 0.0001$ vs Control.

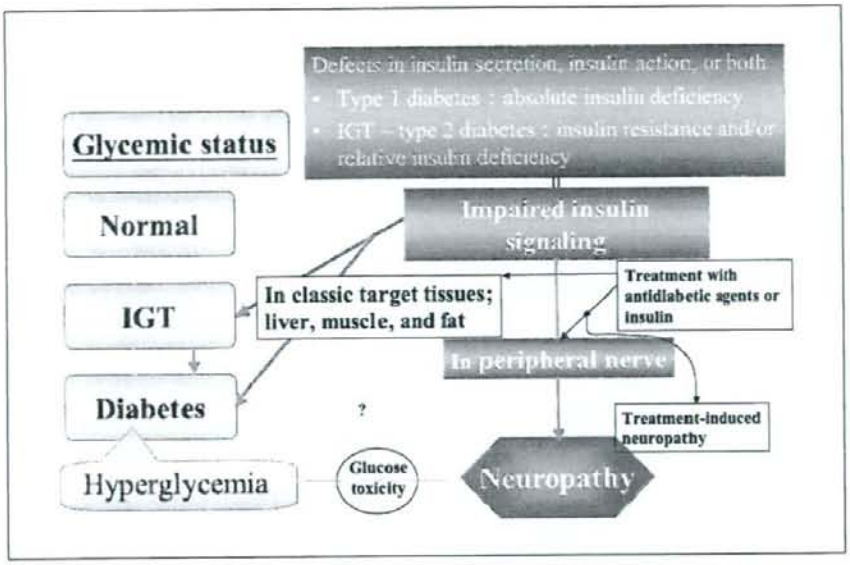

Fig. 4　Putative role of impaired peripheral nerve insulin signaling in the development of diabetic neuropathy.
In this scenario, impaired insulin signaling in peripheral nerves and subsequent neuropathic changes may precede deterioration of glucose tolerance due to impaired insulin signaling in the classic insulin target tissues. Treatment with antidiabetic agents or insulin may further aggravate impairment of insulin signaling in peripheral nerves in treatment-induced neuropathy.
IGT, impaired glucose tolerance.

1型糖尿病ラットモデルにおける痛覚反応と末梢神経内インスリン受容体リン酸化の経時変化

糖尿病性神経障害の基礎的研究に汎用される代表的1型糖尿病動物モデルであるSTZ-Dラットでは，前述のように糖尿病誘発後直ちに神経機能の異常を示す。Fig. 3に，このラットのTFLと後肢の機械的刺激に対する反応域値（PWT, paw withdrawal threshold）の経時変化を示す。このモデルでは，糖尿病誘発後1–2週目までに温痛覚鈍麻（Fig. 3-A）と機械的痛覚過敏（Fig. 3-B）が出現し，以降これらの痛覚異常に悪化傾向は認められない。高血糖に影響しない微量のインスリン補充が，このモデルの痛覚異常を改善することが示されている[8]。最近我々は，このモデルの末梢神経において，総インスリン受容体β蛋白に対するチロシンリン酸化インスリン受容体の発現比が，糖尿病誘発2週目までの早期から低下することを報告している（Fig. 3-C）[9]。この早期からの末梢神経内インスリン受容体シグナルの変化は，慢性高血糖によるものではなく，このモデルに見られる急激なインスリン欠乏症によるものと捉えるべきであろう。今後，この変化が，このモデルの急性かつ非進行性の神経機能障害の成因にいかなる役割を果たしているのか，より詳細な検索が必要と考える。

インスリン分泌・作用異常から見た糖尿病性神経障害

Thrainsdottirら[10]は，6年間の追跡調査において，追跡開始時に耐糖能正常あるいはIGTで，のちにIGTあるいは糖尿病へ耐糖能の悪化を示した例は，悪化を示さなかった例に比べて，神経内細小血管密度の上昇と内腔の

狭小化を示すことを報告している。このことは，糖尿病性神経障害の特徴である神経内細小血管障害が，IGTや糖尿病の発症に先駆けて生じていることを示唆している。確かに，糖尿病性神経障害の成因に，高血糖が重要な役割を持つことは確立された事実である。しかしながら，糖尿病性神経障害の病因機序を，前述したIGTに関連する神経障害や治療後神経障害を含め，包括的に理解しようとする時，高血糖をその中心に据えた「高血糖仮説」のみで説明することは困難とも言える。この際，筆者は，糖尿病そのものの主因がインスリンの分泌，作用，あるいはその両方の異常によってもたらされる肝，筋そして脂肪組織と言った古典的インスリン標的臓器におけるインスリンシグナル異常であるように，糖尿病に特異的に合併する神経障害も，末梢神経におけるインスリンシグナル異常に起因すると推定している（**Fig. 4**）。

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RESEARCH ARTICLE

Time course of pain sensation in rat models of insulin resistance, type 2 diabetes, and exogenous hyperinsulinaemia

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Abstract

Background Small sensory fibre dysfunction has been recently recognized as a component of impaired glucose tolerance and insulin resistance (IR) syndrome. However, few studies have investigated whether small sensory fibre dysfunction develops in normoglycaemic or pre-diabetic animal models of IR and/or hyperinsulinaemia. In addition, scant information is available on the metabolic features of IR in relation to small sensory fibre dysfunction due to the progressive failure of beta cells to compensate for IR during the development of frank diabetes.

Methods Longitudinal trends for thermal and mechanical nociceptive responses were assessed in 8–36-week-old male obese Zucker rats, 8–36-week-old male Zucker diabetic fatty (ZDF) rats, and 10–39-week-old male Wistar rats that continued to receive exogenous insulin (2–4 U/day) from subcutaneously implanted insulin pellets. Data were compared with the metabolic disorders in these rats.

Results Both obese Zucker and ZDF rats at 8 weeks of age showed compensatory hyperinsulinaemia and developed thermal hyperalgesia prior to the onset of overt hyperglycaemia. These animals also exhibited progression from thermal hyperalgesia to hypoalgesia, which occurred more rapidly and coincided with a more rapid decline in pancreatic insulin secretion in ZDF rats than in obese Zucker rats. Non-diabetic rats treated with insulin tended to show thermal and mechanical hypoalgesia that was detectable 12–20 weeks after treatment.

Conclusion In addition to insulin treatment, IR with or without compensatory hyperinsulinaemia is associated with nociceptive dysfunction of different phenotypes, independent of glycaemic levels. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords insulin resistance; hyperinsulinaemia; small sensory fibre neuropathy; behavioural test; nociception; adipokines

Introduction

Diabetic neuropathy often presents as the initial clinical manifestation of diabetes and may even precede the onset of diabetes [1–3]. Recent studies have indicated that the pre-diabetic state of impaired glucose tolerance (IGT) is associated with painful sensory neuropathy [4–7] that is indistinguishable from the typical phenotype of early diabetic neuropathy, with the predominant

involvement of small sensory fibres [8–10]. Such findings may give rise to the view that factors other than chronic hyperglycaemia contribute to the development of neuropathy similar to early diabetic neuropathy [11,12]. IGT is one component of metabolic syndrome [13], which consists of a cluster of specific metabolic features of insulin resistance (IR) including central obesity, hypertension, dyslipidaemia, and dysregulation of adipokine secretions [14,15].

A recent study demonstrated significantly higher serum insulin and triglyceride levels with greater IR in patients with painful neuropathy than in control subjects after adjusting for age, sex, and body mass index [16]. Other investigators [17] have reported that lifestyle intervention with aggressive diet and exercise counselling in patients with IGT and neuropathy results in epidermal nerve fibre reinnervation and pain improvement, in addition to reductions in weight, 2-h post-load glucose during oral glucose tolerance testing, and total serum cholesterol.

Kamiya *et al.* [18] demonstrated a significant difference in the deterioration of thermal nociceptive dysfunction between type 1 and type 2 diabetic rats exposed to hyperglycaemia at the same duration and severity. These authors suggested that impaired insulin action was more pertinent to the pathogenesis of small fibre dysfunction than hyperglycaemia. However, no information is available on the duration of time between the onset of IGT and small sensory fibre function deterioration. Additionally, information is required on the metabolic features of IR related to small sensory fibre dysfunction due to the progressive failure of beta cells to compensate for IR during the development of frank diabetes. Such information would be particularly useful for the identification of individuals with IGT who are at a risk of developing diabetic neuropathy and for the establishment of an effective strategy to prevent and ameliorate this condition.

The obese Zucker rat, an animal model with metabolic properties similar to metabolic syndrome with obesity-related IR [19,20], is characterized by early-onset obesity, hyperlipidaemia, and hyperinsulinaemia, with only mild-to-moderate hyperglycaemia that persists throughout its life. The pancreatic beta cells of the obese Zucker rat have a robust, lifelong insulin-secreting capacity that compensates for IR [21]. In contrast, the Zucker diabetic fatty (ZDF) rat, a substrain of the obese Zucker rat, is an established model for type 2 diabetes in which hyperglycaemia initially manifests at about 7 weeks of age. Although the blood insulin level in the ZDF rat increases until 10 weeks of age, the level subsequently drops with the progressive failure of beta cells to compensate for IR, which leads to frank hyperglycaemia and short-term obesity [22,23].

Although these two rat models of IR exhibit different degrees of hyperglycaemia, which reflect different insulin-secreting capacities, they both possess a homozygous mutation in the leptin receptor gene (fa/fa) [24] that impairs the ability of leptin to suppress food intake, thereby resulting in hyperphagia and obesity. Therefore, these models allow us to explore, independently of genetic background, the relationship between metabolic disorders and small sensory fibre dysfunction associated with IR in the presence or absence of frank diabetes and hyperinsulinaemia.

There is evidence that insulin acts as a potent neurotrophic factor for neuronal cells [25]. Insulin has been shown to specifically stimulate neurite outgrowth and regeneration of sensory neurons [26,27]. We reported previously that high-affinity insulin receptors are expressed preferentially in small-to-medium-sized sensory neurons in rats [28,29] and that chronic hyperinsulinaemia in rats results in increased small myelinated axons [30]. However, no study has examined the effect of long-term administration of insulin on small sensory fibre function in normal rats to determine whether chronic hyperinsulinaemia *per se* contributes to small sensory fibre dysfunction, independent of IR.

In the present study, we therefore attempted to characterize longitudinal trends in small sensory fibre function as assessed by behavioural responses to noxious thermal and mechanical stimuli in the obese Zucker rat, ZDF rat, and Wistar rat with chronic exogenous hyperinsulinaemia induced by the subcutaneous administration of insulin. These findings were compared with the metabolic disorders, particularly with changes in circulating insulin levels found in these three models during a prolonged observation period of 8 months.

Materials and methods

Experimental animals

Male obese Zucker rats (Crlj:ZUC-fa/fa) ($n = 22$) were used as a model of obesity-associated IR; age- and sex-matched lean Zucker rats (Crlj:ZUC-+/?) ($n = 22$) served as controls (Charles River Laboratories Japan, Tokyo, Japan). Male ZDF rats (ZDF/GmiCrl-fa/fa) ($n = 60$) were used as a model of insulin-resistant type 2 diabetes; age- and sex-matched lean ZDF rats (ZDF/GmiCrl-+/?) ($n = 27$) served as controls (Charles River Laboratories, Portage, MI, USA). Non-diabetic male Wistar rats ($n = 16$) (Clea Japan, Tokyo, Japan) were divided into two groups of eight animals each. One group was treated with insulin via a sustained-release insulin implant (2 mm × 7 mm) (Linplant; LinShin Canada, Inc., Scarborough, Ontario, Canada) that released regular insulin at a constant rate of ~2 U per 24 h for >40 days; the other group was untreated and served as the control group. Insulin-treated rats received approximately 1 U of regular insulin per 150 g of body weight per 24 h and were used as a model of exogenous hyperinsulinaemia. This insulin dosage regimen has been shown to produce mild to moderate hyperinsulinaemia without severe hypoglycaemia [31,32] or IR [33].

In the insulin-treated rats, one implant was placed in the subcutaneous tissue located on the back when the rats were 10 weeks of age (body weight, 305 ± 4 g; $n = 8$).

Under ether anaesthesia, this implant was replaced every 6 weeks with 1.5 implants until 22 weeks of age (body weight, 497 ± 13 g; $n = 7$). Thereafter, two implants were placed until 40 weeks of age (body weight, 594 ± 19 g; $n = 6$). To minimize hypoglycaemic episodes, insulin-treated rats were given drinking water that contained 10% sucrose for the first 3 days after each new implant. In insulin-treated rats, two animals were not included in the subsequent analysis because one died, and the other developed rapid weight loss of unknown origin.

Each animal's body weight and blood glucose levels were monitored regularly. Whole blood glucose levels of tail vein samples obtained between 14:00 and 16:00 were measured with an Accu-Chek Compact Plus blood glucose meter (Roche Diagnostics K.K., Tokyo, Japan). From each rat group, a subset of non-fasting animals of the indicated age was sacrificed by exsanguination from the left cardiac ventricle under deep anaesthesia with pentobarbital (~100 mg/kg) between 10:00 and 16:00 to obtain blood samples for the determination of haemoglobin A_{1c}, serum lipid, insulin, adiponectin, and leptin levels with a Dimension clinical chemistry system (Dade Behring Inc., Newark, DE, USA), a TBA-200 FR automatic analyzer (Toshiba Medical Systems, Tokyo, Japan), a rat insulin ELISA kit (Linco Research Inc., St. Charles, MO, USA), a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), and a rat leptin ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), respectively. Total cholesterol and triglyceride levels were measured with commercially available kits (Determiner-L TC II, Kyowa Medex Co., Ltd., Tokyo, Japan and Lipidos Liquid, Toyobo, Co., Ltd., Tokyo Japan, respectively).

All animals were housed in sawdust in plastic cages. Rats were maintained on a 12 h–12 h, light–dark cycle at 22 ± 2 °C and 55 ± 5% relative humidity and allowed free access to tap water and standard laboratory chow (protein, 23.3 wt%; carbohydrate, 57.7 wt%; fat, 5.3 wt%) (MF; Oriental Yeast Co., Ltd., Tokyo, Japan). All animal protocols were approved by the Animal Research Committee and the guidelines for animal experimentation of Hirosaki University were followed.

Behavioural tests of nociception

Before starting the experiments, the animals were allowed to acclimatize to handling-related stress for at least 1 week. Then, the experimental procedures described below were carried out. Thermal sensitivity was assessed with a Tail Flick Analgesymeter (MK-330B; Muromachi Kikai Co., Ltd., Tokyo, Japan). The animal was wrapped gently in a towel and placed on top of the instrument with the tail in the sensing groove. The tail flick latency (TFL) was determined by exposing the tail to a radiant heat source and recording the time taken to remove the tail from the noxious thermal stimulus. The radiation intensity was chosen based on the intensity required to elicit a basal tail flick response of 5.0–8.5 s in untreated Wistar rats. For each animal, two or three recordings were made at an interval of >15 min; the mean value was used for statistical analysis.

The mechanical nociceptive threshold (MNT) was assessed with the Randall-Selitto test using an Analgesymeter (Ugo-Basile, Varese, Italy). A constantly increasing pressure stimulus (with increase at a rate of 16 g/s) was applied to the dorsal surface of the hind paw of the rat while the animal was gently restrained under a soft towel. To avoid tissue damage, a cut-off of 250 g was used. The pressure was increased until the animal withdrew the paw, squeaked, or struggled. One measurement per paw was performed with an interval of >15 min between measurements. For each animal, the results from both the paws were averaged for statistical analysis.

Data analysis

All statistical analyses were conducted with Stat View software (version 4.5) and SPSS (version 16.0) for Macintosh. Values are reported as the means ± SE. The significance of differences in mean values between the two groups was tested by ANOVA followed by the Bonferroni/Dunn test. Age-adjusted partial correlation coefficients between serum insulin levels and the TFL and MNT in lean Zucker, obese Zucker, lean ZDF, and ZDF rats of different ages were calculated after data with significantly skewed distributions were transformed logarithmically. A two-sided P value <0.05 was considered statistically significant.

Results

Body weight

Obese Zucker rats developed early-onset and sustained obesity. At 8 and 36 weeks of age, the obese Zucker rats had 31 and 50% greater body weight than lean Zucker rats, respectively (Figure S1, see Supporting Information). ZDF rats developed early-onset and short-term obesity. At 8 weeks of age, ZDF rats had a 27% greater body weight than lean ZDF rats, which persisted up to 14 weeks of age, and then they lost some weight after 24 weeks of age (Figure 1(B)). Compared with untreated control rats, insulin-treated rats exhibited a progressive increase in body weight. Insulin-treated rats had a 6% greater body weight at 2 weeks and were 14% heavier at 30 weeks after insulin administration (Figure 1(C)).

Glycaemia

Obese Zucker rats were normoglycaemic at 8 weeks of age. After 10 weeks of age, they developed mild hyperglycaemia, with mean non-fasting blood glucose levels ranging from 140 to 220 mg/dL (Figure 1(D)).

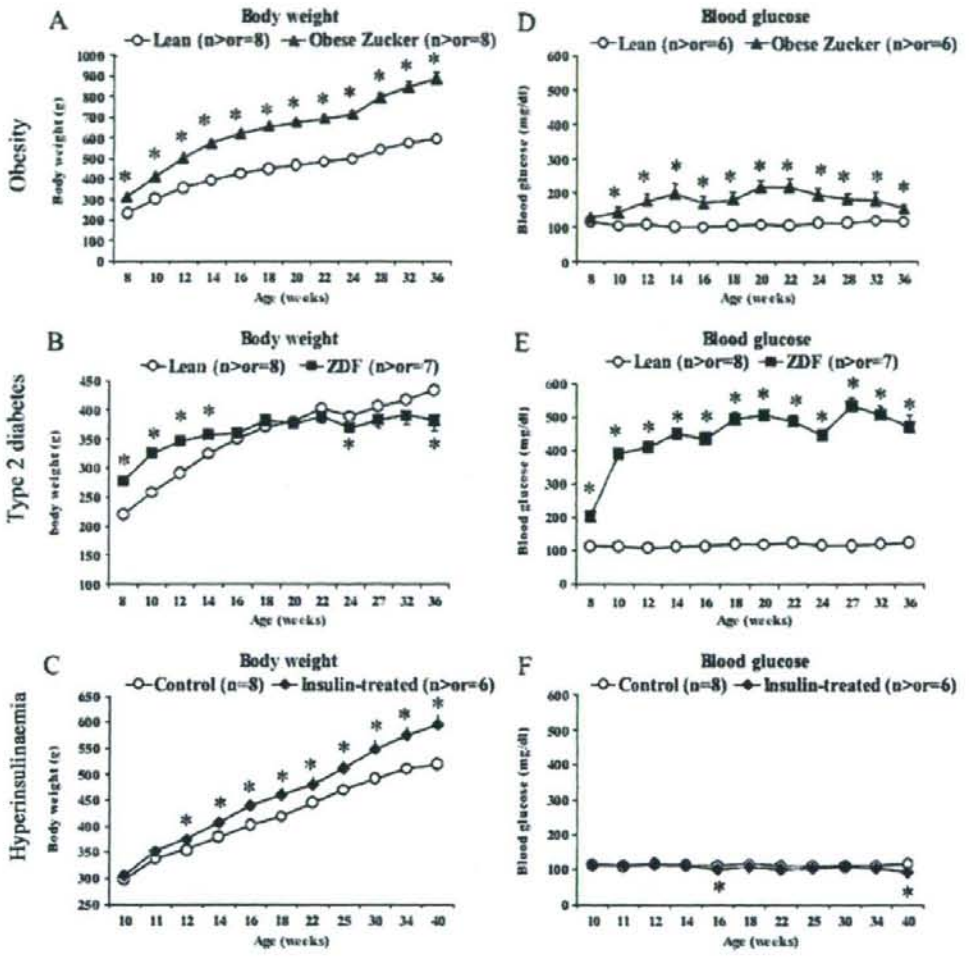

Figure 1. Time course of changes in body weight (A-C) and blood glucose (D-F) in the rat models of insulin resistance (A, D), type 2 diabetes (B, E), and hyperinsulinaemia (C, F). Data are means ± SE. Most bars are not seen because of the small SE. $^*P < 0.05$ *versus* the age-matched control group (ANOVA)

In this obese model, A_{1c} levels were similar to those in lean Zucker rats at 8 weeks of age. Compared with lean Zucker rats, the A_{1c} levels in obese Zucker rats increased by 37% at 24 weeks of age and 21% at 38 weeks of age (Table 1). The 8-week-old ZDF rats showed mild hyperglycaemia (204 ± 12 mg/dL) that rapidly progressed such that the mean non-fasting blood glucose level reached >400 mg/dL after 12 weeks of age (Figure 1(E)). Compared with lean ZDF rats, the A_{1c} levels in this type 2 diabetes model increased by 59% at 10 weeks, 136% at 24 weeks, and 150% at 39 weeks of age (Table 1). Insulin-treated rats occasionally showed a mild to moderate reduction in the non-fasting blood glucose level, which was >45 mg/dL during the 30-week observation period (Figure 1(F)). A_{1c} levels in this hyperinsulinaemia model were decreased by 5% at 40 weeks of age compared with untreated rats (Table 1).

Serum insulin

Obese Zucker rats developed hyperinsulinaemia which slowly decreased with age. Compared with lean Zucker rats, a significant 25-fold increase in serum insulin levels was seen at 8 weeks, a significant 3.9-fold increase was seen at 24 weeks, and an insignificant 1.9-fold increase was seen at 38 weeks (Figure 2(A)). ZDF rats developed early-onset/short-term hyperinsulinaemia. Compared with lean ZDF rats, these rats exhibited a significant 9.7-fold increase in serum insulin levels at 10 weeks and equivalent serum insulin levels at 24 weeks and 39 weeks of age (Figure 2(B)). Insulin-treated rats at 40 weeks of age had a significant 2-fold increase in serum insulin levels compared with untreated control rats (Figure 2(C)).

Table 1. Laboratory data for each rat model at different ages

Rat model	*n*	Age	A_{1c} (%)	Adiponectin (μg/mL)	Leptin (ng/mL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)
Obesity-associated insulin resistance							
Lean	6	8 weeks	2.7 ± 0.0	4.3 ± 0.7	3.2 ± 0.6	77.8 ± 2.3	81.7 ± 12.9
Obese Zucker	6		2.8 ± 0.1	11.4 ± 1.1*	44.9 ± 3.1*	115.5 ± 4.9*	350.8 ± 50.9*
Lean	8	24 weeks	4.1 ± 0.1	2.6 ± 0.3	5.7 ± 0.7	94.1 ± 5.8	154.4 ± 33.3
Obese Zucker	8		5.6 ± 0.4*	4.1 ± 0.4*	33.8 ± 6.7*	249.9 ± 36.6*	1467.6 ± 419.7*
Lean	8	38 weeks	4.2 ± 0.0	2.7 ± 0.2	8.1 ± 1.1	177.9 ± 25.7	418.0 ± 98.9
Obese Zucker	8		5.1 ± 0.2*	5.8 ± 0.8*	70.8 ± 26.4*	322.9 ± 23.3*	1404.4 ± 253.8*
Type 2 diabetes							
Lean	6	10 weeks	3.9 ± 0.0	11.4 ± 2.2	3.1 ± 0.5	61.5 ± 1.5	41.2 ± 5.1
ZDF	6		6.3 ± 0.1*	9.4 ± 0.5	25.2 ± 1.8*	116.0 ± 5.1*	695.3 ± 88.1*
Lean	12	24 weeks	3.6 ± 0.1	4.8 ± 0.2	4.1 ± 0.6	91.7 ± 2.4	82.3 ± 7.3
ZDF	13		8.5 ± 0.2*	5.7 ± 0.6	8.3 ± 0.8*	197.1 ± 9.2*	440.4 ± 58.4*
Lean	8	39 weeks	3.4 ± 0.1	6.5 ± 0.2	5.2 ± 0.4	91.0 ± 1.8	87.0 ± 7.4
ZDF	7		8.5 ± 0.2*	6.8 ± 0.3	8.8 ± 0.9*	266.6 ± 29.2*	747.6 ± 189.5*
Exogenous hyperinsulinaemia							
Untreated	8	40 weeks	3.7 ± 0.0	–	–	83.4 ± 2.8	241.3 ± 22.2
Insulin-treated	6		3.5 ± 0.1*	–	–	84.8 ± 4.9	217.2 ± 16.4

Data are means ± SE. *Significantly different ($P < 0.05$) from the age-matched control group.

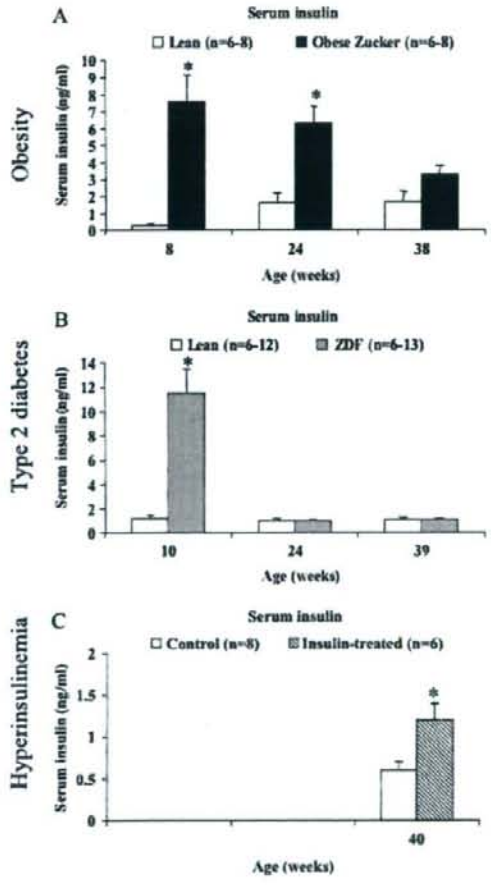

Figure 2. Serum insulin levels as a function of age in the rat models of insulin resistance (A), type 2 diabetes (B), and hyperinsulinaemia (C). Data are means ± SE. *$P < 0.05$ *versus* the age-matched control group (ANOVA)

Serum adipokines

Serum levels of adiponectin and leptin in obese Zucker and ZDF rats are shown as a function of age in Table 1. At all three time points, obese Zucker rats had significant 1.6–2.7-fold increases in serum adiponectin levels and significant 6-14-fold increases in serum leptin levels compared with lean Zucker rats. Compared with lean ZDF rats, ZDF rats had similar serum adiponectin levels and significant 1.7–8.1-fold increases in serum leptin levels at all three time points.

Serum lipids

Serum total cholesterol and triglyceride levels in obese Zucker, ZDF, and insulin-treated rats are shown as a function of age in Table 1. Compared with lean Zucker rats, obese Zucker rats had significant 1.5–2.7-fold increases in serum total cholesterol levels and significant 3.4–9.5-fold increases in serum triglyceride levels at all three time points. Compared with lean ZDF rats, ZDF rats had significant 1.9–2.9-fold increases in serum total cholesterol levels and significant 5.4–16.9-fold increases in serum triglyceride levels at all three time points. Insulin-treated rats had serum total cholesterol and triglyceride levels that were comparable with those of the untreated control rats at 40 weeks of age.

Behavioural thermal nociception

Obese Zucker rats developed thermal hyperalgesia, as indicated by a significant 21% decrease in the TFL at 8 weeks of age. They later developed thermal hypoalgesia, as shown by significant 27-36% increases in the TFL after 32 weeks of age (Figure 3(A)). ZDF rats developed thermal hyperalgesia, with a significant 15% decrease in the TFL at 8 weeks of age. ZDF rats also developed

thermal hypoalgesia, as indicated by significant 21-37% increases in the TFL after 16 weeks of age (Figure 3(B)). Insulin-treated rats temporarily developed thermal hypoalgesia, with significant 9–18% increases in the TFL 12–16 weeks after insulin administration (Figure 3(C)).

Behavioural mechanical nociception

The MNT in response to a noxious pressure stimulus remained unaltered in obese Zucker rats aged 8–36 weeks (Figure 3(D)). ZDF rats developed mechanical hyperalgesia, as indicated by significant 22–36% decreases in the MNT after 18 weeks of age (Figure 3(E)). There was a trend which did not reach statistical significance, for the MNT to increase in insulin-treated rats 6–20 weeks after insulin administration (Figure 3(F)).

Serum insulin levels and behavioural nociceptive responses

A significant partial correlation coefficient between log-transformed serum insulin levels and the TFL was

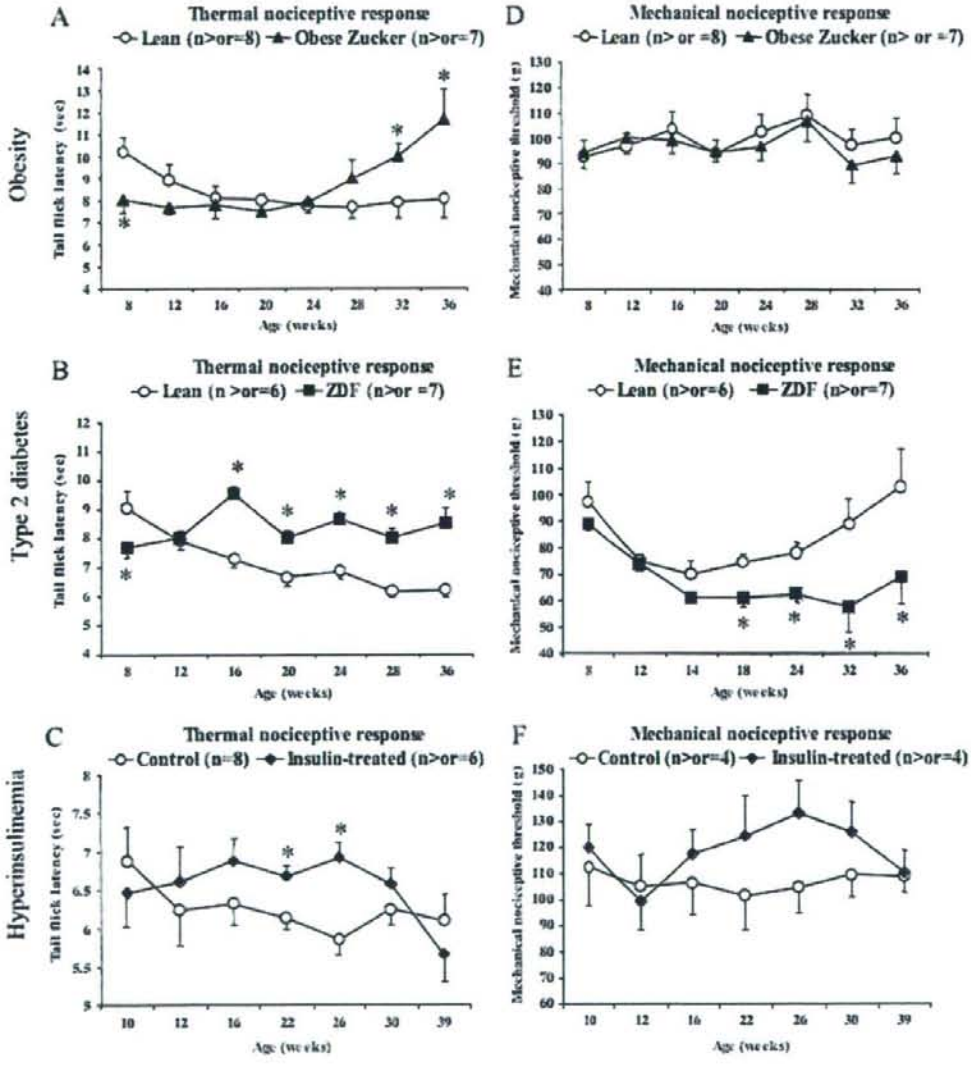

Figure 3. Time course of changes in tail flick latency (A-C) and mechanical nociceptive threshold (D-F) in rat models of insulin resistance (A, D), type 2 diabetes (B, F), and hyperinsulinaemia (C, F). Data are means ± SE. *$P < 0.05$ *versus* the age-matched control group (ANOVA)

observed only in ZDF rats after adjustment for age ($r = -0.456$, $P = 0.022$), whereas there was no significant correlation between serum insulin level and the MNT in any rat group (Figure S2, see Supporting Information).

Discussion

The present study demonstrated that 8-week-old, normoglycaemic, obese Zucker and mildly hyperglycaemic ZDF rats exhibited transient thermal hyperalgesia that was associated with the characteristic metabolic features of IR including obesity, hyperlipidaemia, hyperleptinaemia, and compensatory hyperinsulinaemia. Earlier studies demonstrated that female obese Zucker rats with fasting normoglycaemia, aged 17–21 weeks, developed thermal hyperalgesia as indicated by a decreased TFL [34]. In addition, in ZDF rats, thermal hyperalgesia, as indicated by a decrease in paw fanning or licking latency to a noxious thermal (hot plate) stimulus, occurred by 9 weeks of age and was not prevented by the insulin sensitizer rosiglitazone which maintained normoglycaemia [35]. Collectively, these findings suggest that early nociceptive dysfunction is characterized by thermal hyperalgesia and is independent of chronic hyperglycaemia in rat models of IR.

In addition, thermal hyperalgesia progressed to thermal hypoalgesia in obese Zucker rats after 32 weeks of age and in ZDF rats after 16 weeks of age in the presence of sustained hyperglycaemia, hyperlipidaemia, and hyperleptinaemia after 10 weeks of age. It has been reported that the Otsuka Long-Evans Tokushima fatty (OLETF) rat, a model of spontaneous type 2 diabetes, develops thermal hyperalgesia, as indicated by a decrease in paw withdrawal latency to noxious thermal stimulus, at 9–14 weeks of age [36] and thermal hypoalgesia, as indicated by an increase in TFL, after 10 months of age [37]. In this model, plasma insulin responses to glucose and glucagon are higher by 16–24 weeks of age, but are lower after 40 weeks of age than those in age-matched, non-diabetic control rats [38].

In the present study, a more rapid progression from thermal hyperalgesia to hypoalgesia appeared to coincide with a more rapid decline in pancreatic insulin secretion in ZDF rats than in obese Zucker rats (Figure 2(A) and (B)). Therefore, in rat models of IR and type 2 diabetes, thermal hyperalgesia coincides with increased pancreatic insulin secretion, whereas thermal hypoalgesia coincides with impaired pancreatic insulin secretion to compensate for IR. This finding is in agreement with a recent report [18] suggesting that impaired insulin action is more pertinent to the pathogenesis of diabetic nociceptive dysfunction than hyperglycaemia. Interestingly, we found a significant negative correlation between serum insulin levels and the TFL after adjustment for age in ZDF rats. However, to establish whether this association is causal and to explore the associated mechanisms, future studies should include more detailed measurements and adjustment for potential confounding factors such as IR.

In the present study, mechanical hyperalgesia was observed only in ZDF rats after 18 weeks of age. It has been reported that 13-week-old ZDF rats developed mechanical hyperalgesia as indicated by a decrease in the MNT, which was ameliorated by low-dose insulin-like growth factor (IGF)-II treatment in the presence of chronic hyperglycaemia [39]. Furthermore, in the streptozotocin-induced diabetic rat, a model of type 1 diabetes, low-dose insulin treatment can ameliorate mechanical hyperalgesia without affecting glycaemic levels [40,41]. We reported previously that in the STZ-diabetic rat, mechanical hyperalgesia occurs concurrently with the rapid impairment of peripheral nerve insulin signalling that is detectable immediately after the onset of diabetes [42]. These findings suggest a role for insulinopenia, rather than chronic hyperglycaemia, in impaired peripheral nerve insulin signalling and the pathogenesis of nociceptive dysfunction in the rat model of type 1 diabetes [43]. Thus, it is possible that in ZDF rats, severe IR and the progressive decline of pancreatic insulin secretion might be involved in nociceptive dysfunction.

During the long-term observation periods, serum adiponectin levels were increased in obese Zucker rats but were unchanged in ZDF rats compared with the respective age-matched lean rats. The present findings are consistent with previous results for obese Zucker [44] and ZDF rats [45]. The reason for the difference in circulating adiponectin levels between the two rat models with the same genetic background is unknown. However, these findings suggest that, despite its pivotal role in the development of atherosclerosis [46], adiponectin is unlikely to be involved in the development of nociceptive dysfunction in rat models of IR and type 2 diabetes.

In contrast to compensatory hyperinsulinaemia in rat models of IR and type 2 diabetes, exogenous hyperinsulinaemia in non-diabetic rats may be associated with thermal and mechanical hypoalgesia which occurred 12–20 weeks after insulin treatment. Therefore, it is possible that hyperinsulinaemia in the presence or absence of IR has different effects on nociceptive properties in rats.

It has been demonstrated that exogenous insulin has a hypoxic effect on normal rat peripheral nerves under non-hypoglycaemic conditions [47]. Local insulin administration has been reported to have a trophic influence on myelinated fibres, which is independent of hyperglycaemia in rats [48]. We have found that rat peripheral nerves bear high-affinity and highly phosphorylated insulin receptors, which are predominantly located in the nodal and paranodal apparatus, endoneurial microvessels, and small-to-medium-sized sensory neurons that give rise to nociceptive primary afferent fibres [28,29,42]. Therefore, it can be hypothesized that insulin exerts its regulatory effects on endoneurial microcirculation, regeneration, and nociception via interaction with its receptor, independent of its hypoglycaemic effect.

In summary, the present study demonstrated the progression from transient thermal hyperalgesia to thermal

hypoalgesia in rat models of IR and type 2 diabetes during an extended observation period of >8 months. The relationships between the observed nociceptive dysfunction and metabolic disorders suggest that thermal hyperalgesia is associated with early metabolic disorders of IR with compensatory hyperinsulinaemia. Furthermore, thermal hypoalgesia appears to be associated with impaired insulin secretion to compensate for IR in these models. In addition, chronic exogenous hyperinsulinaemia may be associated with thermal and mechanical hypoalgesia in non-diabetic rats. Thus, we conclude that the behavioural responses to thermal and mechanical stimuli may be altered by circulating insulin levels and may be independent of glycaemic levels in rats.

Supporting information

Supporting information may be found in the online version of this article.

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Conflict of interest

None declared.

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