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Effects of NO-1886 on inflammation-associated cytokines in high-fat/high-sucrose/high-cholesterol diet-fed miniature pigs

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Abstract

Inflammation, closely associated with obesity, is emerging as an important risk factor for the pathophysiological development of atherosclerosis and diabetes mellitus. Fat balance is critical in the aetiology of obesity. Lipoprotein lipase is an important enzyme in lipid metabolism. The aim of this study was to investigate the long-term effect of the lipoprotein lipase activator, NO-1886, on inflammation cytokines, adiposity and related diseases in miniature pigs fed a high-fat/high-sucrose/high-cholesterol diet (HFSC diet). Chinese Bama-miniature pigs were fed a control diet or HFSC diet with or without NO-1886 for 5 months. The levels of inflammation-associated cytokines were determined using the antibody arrays. Feeding of the HFSC diet to miniature pigs markedly increased the expression of inflammatory cytokines. On the other hand, supplementation of NO-1886 to HFSC diet decreased the expression of inflammatory cytokines significantly, protecting against the development of atherosclerosis and diabetes mellitus. NO-1886 may have a beneficial effect on the most inflammation-associated cytokines, and this effect may contribute to improving atherosclerosis and diabetes mellitus.

Keywords: NO-1886; Inflammatory cytokine; Atherosclerosis; Diabetes mellitus

1. Introduction

Persistent low-grade inflammation is emerging as an important contributor to the pathogenesis of diabetes mellitus and atherosclerosis (Pickup, 2004; Duncan et al., 2003; Ridker, 2002; Weissberg and Bennett, 1999; Fernandez-Real and Ricart, 2003). Given that widespread deleterious health effects are observed in augmented inflammatory state, identification of therapies that reduce inflammation is critical (Nicklas et al., 2005). It is strongly evident that circulating levels of inflammatory markers are elevated with total and abdominal obesity, especially visceral obesity, possibly owing to a higher secretion rate of inflammatory cytokines by adipose tissue in obese people (Esposito et al., 2003; Marfella et al., 2004; Ryan and Nicklas, 2004; Lemieux et al., 2001; Forouhil et al., 2001; Saijo et al., 2004). There are promising data indicating that decreasing energy intake and body weight gain and increasing physical activity may be effective in reducing overall inflammation (Esposito et al., 2003; Marfella et al., 2004; Ryan and Nicklas, 2004).

It was reported previously that a synthetic compound, NO-1886, had a potent lipoprotein lipase-enhancing activity (Tsutsumi et al., 1996; Hagi et al., 1997). Kusunoki et al. (2000) reported that NO-1886 inhibited fat accumulation in high-fat-induced obesity diabetes type 2 animal model rats. Recently, Yin has reported that NO-1886 decreased body weight and ectopic lipid deposition (Yin et al., 2004a,b), reducing size of

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adipocytes, suppressing plasma tumor necrosis factor-α (TNF-α), and decreasing free fatty acids levels in miniature pigs that received a high-fat/high-sucrose diet (Yin et al., 2004a,b). These results suggest that long-term administration of NO-1886 may have a beneficial effect on inflammatory factors. The observations on inflammatory cytokines in the present study are interesting and no studies are reported on the effect of NO-1886 up to date. The aim of this study was to clarify the effect of the compound on inflammation cytokines in miniature pigs with diabetes mellitus and atherosclerosis induced by feeding HFSC diet.

2. Materials and methods

2.1. Reagents

NO-1886 ([4-(4-Bromo-2-cyano-phenylcarbamoyl)-benzyl]-phosphonic acid diethyl ester, CAS 133208-93-2; Lot. No. C00C99; see Fig. 1 for structure formula) was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory Inc. Tokushirrm Japan. Sucrose was obtained from Liuzhou sugar Co. (Guangxi, China), lard was obtained from Hengyang Meat Product Co. (Hunan China). Cholesterol was obtained from Sigma (>99% pure; St. Louis, MO).

2.2. Animal preparation and treatment

Fifteen male Chinese Bama-miniature pigs, 3 to 4 months of age, were obtained from the barrier unit at the Laboratory Animal Center of Third Military Medical University (Chongqing, China). Animals were randomized into three groups with similar body weight [$n=5$ in the normal control diet group, $n=5$ in the high-fat/high-sucrose/high-cholesterol diet (HFSC diet) group, and $n=5$ in the HFSC diet group supplemented with NO-1886 (HFSC diet+NO-1886 group)].The HFSC diet used in this study was the control diet supplemented with 10% lard, 37% sucrose, and 2% cholesterol (the composition is shown in Table 1), which was similar to a "diabetogenic" or "atherogenic" diet (Yin et al., 2002; Finking and Hanke, 1997). Animals were housed in single pens under controlled conditions (temperature between 18 °C and 22 °C, relative air humidity between 30% to 70%, with four times of air changes per hour). Animals were fed three times daily on a restricted feeding schedule (at 8.00 am, 12.00 am, and 6.00 pm) with a control diet, HFSC diet, or HFSC diet+NO-1886. All the animals were given the same amount and carefully observed. During the study period, their appetites were good and dejectas were normal. The total study period was

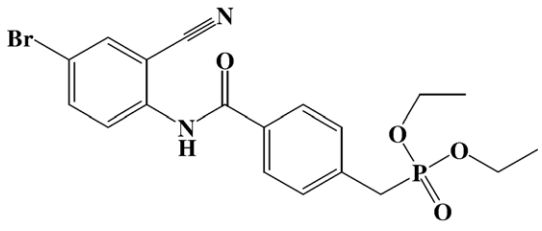

Fig. 1. Chemical structure of NO-1886 (ibrolipim).

Table 1
The ingredients and nutritive values of diets

Components	Control diet (%)	High-fat/high-sucrose/high-cholesterol diet (%)
Rice	64.11	31.68
Wheat bran	10.51	5.35
Soybean meal	11.98	6.11
Cottonseed meal	4	2.03
Colza meal	4	2.03
Fish powder	2	1
Bone powder	1.1	0.5
Calcium bicarbonate	0.8	0.8
Salt	0.5	0.5
Trace elements	0.5	0.5
Vitamins	0.5	0.5
Pork lard		10
Sucrose		37
Cholesterol		2
Crude protein (%)	16.21	8.14
Crude fat (%)	3.61	11.55
Crude fibre (%)	3.09	1.56
Digestible energy (MJ/Kg)	13.13	16.55

5 months. Body weights were recorded every two weeks. Blood samples for plasma parameters were withdrawn from the orbital sinus at the end of each month following an overnight fast. The animals were sacrificed at the end of month 5 for histological preparations and comparison of fat tissue weight. The abdominal subcutaneous adipose tissues were dissected from adjacent tissues and frozen in liquid nitrogen. The institutional guidelines of Nanhua University for animal care and use were followed. The local animal ethics committee of Nanhua University approved the conduct of this study.

2.3. Measurement of plasma parameters

Lipid plasma parameters were determined according to previous methods (Yin et al., 2004a,b, 2002). Plasma C-reactive protein (CRP-test kit) was determined by latex-based immunoassay (Rongsheng Biotech Inc., Shanghai, China); plasma plasminogen activator inhibitor-1 (PAI-1) was determined by the enzyme-linked immunosorbent assays (ELISA) (Taiyang Biotech Inc, Shanghai, China).

2.4. Measurement of inflammation cytokines using antibody arrays

Inflammation Antibody Array III (Ray Biotech Inc., Norcross, Ga. Cat# H0128003 Table 3) consisting of 40 different inflammation cytokine antibodies was spotted in duplicate onto a membrane. First, proteins were extracted from fat tissues using 1×Protein Lysis Buffer. After extraction, samples were spun and supernatant was obtained to determine proteins. Then, 2 ml 1×Blocking Buffer was added and incubated at room temperature for 30 min to block array membranes, covering eight-well tray with lid to avoid drying. All washings were handled according to the instructions of the manufacturer. Two milliliters of a 1:500 dilution of biotin-

conjugated antibodies was added to each membrane, and the mixture was incubated on a shaker for an hour and a half at room temperature. After washing, the membranes were incubated with a 1:40,000 dilution of strepavidin-conjugated peroxidase for 1 h at room temperature according to the instructions of the manufacturer. After washing thoroughly, the membranes were exposed to a peroxidase substrate (ChemGlow West; AlphaInnotech Corp., San Leandro, Calif.) for 5 min in the dark before imaging. Three individual membranes were placed side by side in a plastic protective folder and sealed. Imaging was done with a UVP AutoChemi imaging system within 30 min of exposure to the substrate. Exposure times ranged from 1 to 10 min. Chemiluminescence was quantified with LabWorks imaging and analysis software. Horseradish peroxidase (HRP)-conjugated antibody served as a positive substrate control at six spots and was also used to identify membrane orientation. For each spot the net density gray level was determined by subtracting the background gray levels from the total raw density gray levels. The relative fold difference in cytokine amount was determined in reference to the amount present on the control membrane on the basis of the following: average treated cytokine spot gray levels/average control cytokine spot gray levels.

2.5. Measurement of oral glucose tolerance test (OGTT) and insulin sensitivity assay

In order to analyze the effects of NO-1886 on glucose tolerance and insulin sensitivity, we conducted an oral glucose tolerance test and insulin sensitivity test according to previous methods (Yin et al., 2004a,b, 2002).

2.6. Aortic lesion analysis

At the end of the study period, the animals were killed by phlebotomy under light anaesthesia with sodium pentobarbital (30 mg/kg, intravenous, Jilin Northern medicine Inc, China). The aortas were dissected from the heart to the bifurcation and gently rinsed with normal saline, and the periaortic tissue was carefully removed. The arteries were then flattened under a glass plate and photographed. The photograph for each animal was captured into a computer via an image scanner (GT-800; SeikoEpson-Corp., Nagano, Japan) to analyze the surface area of atherosclerotic lesions and of the aorta itself using image analysis software (NIHIMAGE). The aortic lesion area was calculated as a percentage as follows:

$$\frac{\text{Atherosclerotic lesion}}{\text{Area of the aorta}} \times 100$$

2.7. Statistical analysis

Results are expressed as mean±S.D. Comparisons among the 3 groups were analyzed for statistical significance using 1-way analysis of variance, followed by Dunnet's test multiple comparisons. Statistical significance was obtained when P-values were <0.05.

3. Results

3.1. Effect of NO-1886 on body and fat weight

The body weights of the three groups were linearly elevated with time. HFSC diet feeding resulted in a faster increase, whereas supplementing HFSC diet with 1% NO-1886 inhibited body weight gain throughout the study, and significant difference was observed at week 18 and week 20 (Fig. 2A). The amounts of retroperitoneal, epiploon, and mesenteric fats (g/kg body weight) were significantly increased in miniature pigs in the HFSC diet group compared with miniature pigs in the control diet and HFSC diet+NO-1886 groups. NO-1886 decreased abdominal fat accumulation by 42.43% (Fig. 2B).

3.2. Effect of NO-1886 on post-heparin plasma lipoprotein lipase activity

Post-heparin plasma lipoprotein lipase activity (Fig. 3). Specifically, post-heparin plasma lipoprotein lipase activity significantly increased in the HFSC diet group compared to

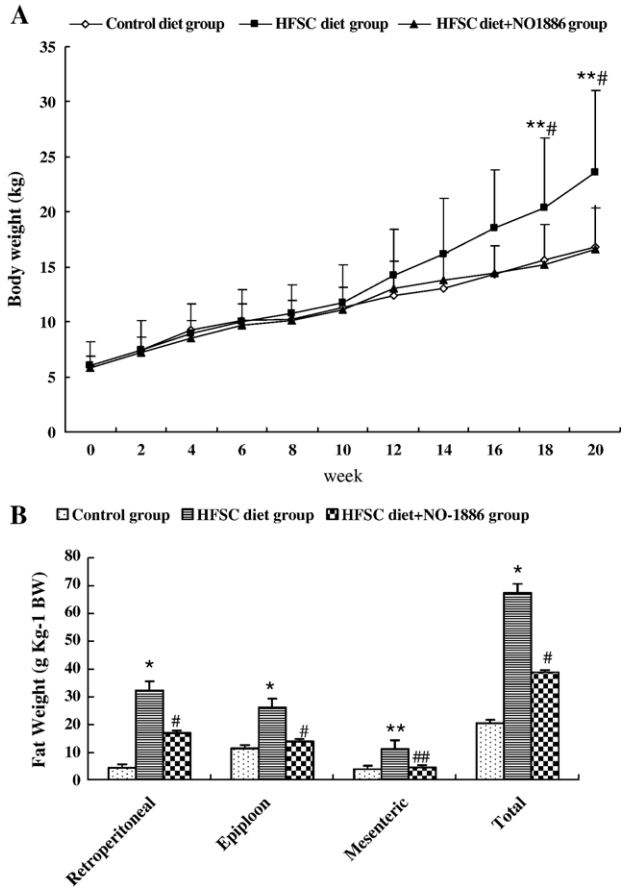

Fig. 2. Changes in the body weight during the 5-month experimental period. Body weight (A) and abdominal fat accumulation (B) at the end of the study was significantly greater in HFSC diet-fed pigs than in control diet and HFSC diet +NO-1886-fed pigs (*$P<0.001$, **$P<0.01$ vs control diet group). NO-1886 inhibited weigh gain and decreased abdominal fat accumulation by 42.43% ($^{\#}P<0.01$, $^{\#\#}P<0.05$ vs HFSC diet group). Each point represents the mean±S.D.

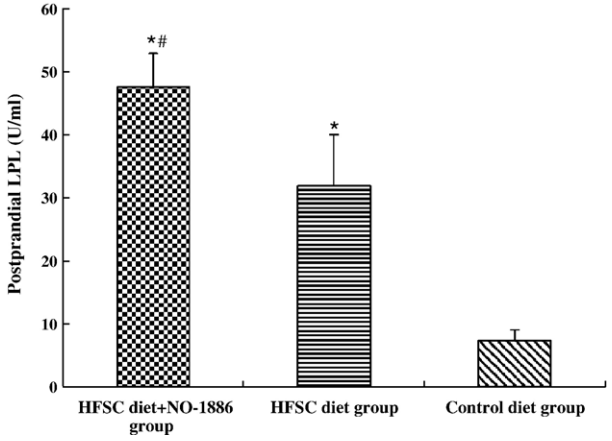

Fig. 3. Effects of control diet or HFSC diet with and without NO-1886 (1.0%) for 5 months on post-heparin plasma lipoprotein lipase activity. *$P<0.05$ vs control diet group; #$P<0.05$ vs HFSC diet group. Each point represents the mean±S.D.

the control diet group. Further, addition of NO-1886 to HFSC diet markedly increased post-heparin plasma lipoprotein lipase activity compared to HFSC group, $P<0.05$ (Fig. 3).

3.3. Effect of NO-1886 on plasma lipid parameters and inflammatory markers

Plasma triglyceride, free fatty acids, glucose, insulin and inflammatory markers C-reactive protein, plasminogen activator inhibitor-1 were significantly increased in miniature pigs that received HFSC diet. The levels of plasma triglyceride, free fatty acids, glucose, insulin, and C-reactive protein in the HFSC diet group were 1.88, 2.13, 1.15, 1.28, 3.33 times of the control, respectively. On the contrary, NO-1886 markedly decreased triglyceride, free fatty acids, glucose, insulin and C-reactive protein, plasminogen activator inhibitor-1 levels (Table 2).

3.4. Inflammatory cytokine antibody array analysis

Most inflammation cytokine expression levels in fat tissue samples significantly increased in the miniature pigs that received HFSC diet compared with the control diet group (Fig. 4). The relative amount of each cytokine spot was shown as the difference between the groups. Relative expression levels of cytokines can be made by comparing the signal intensities. The intensities of signals can be quantified by densitometry (Table 3 presented the significant data). Positive control can be used to normalize the results from different membranes for comparison. Each cytokine is represented by duplicate spots in the specific locations. Among these, A1 and A2, B1 and B2, HRP-conjugated antibody (positive control); C1 and C2, D1 and D2, NEG (negative control group). Inflammation cytokines in HFSC diet group were increased by 52.72% (EOTAXIN-2), 119.00% (G-CSF), 95.00% (ICAM), 132.00% (IFN-γ), 30.80% (I-309), 102.00% (IL-1α), 687.00% (IL-1β), 117.16% (IL-6SR), 37.80% (IL-8), 334.40% (IL-10), 6385.66% (IL-11), 270.88% (IL-12P40), 98.55% (IL-12P70), 87.25% (IL-13), 76.08% (IL-15), 176.96% (IL-16), 574.00% (IL-17), 172.89% (IP-10), 111.28% (MCP-1), 209.00% (MCP-2), 84.3% (MIG), 40.05% (MIP-1δ), 66.73% (RANTES), 47.31% (TGF-β), 25.67% (TNF-α), 33.10% (TNF-β), 58.99% (sTNFRI),

Table 2
Fasting plasma glucose and lipid metabolism in Chinese Bama-miniature pigs fed control diet and HFSC diet and HFSC diet supplement NO-1886

Parameters	0	1 month	2 months	3 months	4 months	5 months
Triglyceride (mmol/l)						
Control diet	0.59±0.04	0.59±0.06	0.63±0.10	0.69±0.16	0.71±0.15	0.73±0.13
HFSC diet	0.59±0.05	0.94±0.07[b]	1.50±0.18[b]	1.87±0.14[b]	2.03±0.42[b]	2.10±0.32[b]
HFSC diet+NO-1886	0.58±0.04	0.60±0.01	0.78±0.08[d]	0.87±0.17[d]	1.03±0.25[d]	1.10±0.35[d]
Free fatty acids (mmol/l)						
Control diet	0.11±0.02	0.23±0.07	0.23±0.06	0.30±0.05	0.29±0.08	0.31±0.04
HFSC diet	0.11±0.03	0.25±0.08	0.28±0.03	0.75±0.24[b]	1.00±0.19[b]	0.97±0.30[b]
HFSC diet+NO-1886	0.15±0.04	0.18±0.06	0.23±0.06	0.37±0.11[d]	0.43±0.12[d]	0.39±0.07[d]
Glucose (mmol/l)						
Control diet	4.71±0.39	4.24±0.90	4.83±0.87	5.24±1.00	4.65±0.71	4.77±0.57
HFSC diet	5.12±0.84	5.97±0.73[a]	6.88±0.57[a]	8.89±2.05[b]	9.54±2.12[b]	10.27±2.25[b]
HFSC diet+NO-1886	4.78±0.62	4.41±1.45	4.96±1.09[c]	5.89±0.50[c]	4.85±1.10[d]	4.75±0.45[d]
Insulin (mU/ml)						
Control diet	6.97±0.33	7.31±0.94	7.50±0.93	8.68±0.82	7.25±0.80	7.65±1.33
HFSC diet	7.47±1.16	9.12±1.63	15.12±3.22[a]	21.16±5.08[a]	24.43±3.55[b]	17.43±3.80[b]
HFSC diet+NO-1886	7.65±0.91	8.73±1.52	9.43±0.84[c]	10.95±2.95[c]	10.30±3.57[d]	10.34±2.71[c]
C-reactive protein (CRP) (mg/l)						
Control diet	1.13±0.35	1.37±0.32	1.50±0.24	1.68±0.68	1.87±0.13	1.77±0.44
HFSC diet	1.57±1.00	2.45±0.29	4.51±0.61[b]	5.13±1.03[b]	6.24±1.21[b]	7.66±0.91[b]
HFSC diet+NO-1886	1.38±0.37	2.02±0.60	3.07±0.80[b/c]	3.35±0.47[b/c]	4.26±0.59[b/c]	4.58±0.66[b/c]
Plasminogen activator inhibitor-1 (PAI-1) (ng/ml)						
Control diet	0.51±0.04	0.50±0.04	0.56±0.04	0.59±0.09	0.65±0.11	0.48±0.04
HFSC diet	0.52±0.06	0.84±0.12	1.30±0.22[b]	1.89±0.28[b]	2.42±0.22[b]	2.52±0.06[b]
HFSC diet+NO-1886	0.51±0.01	0.73±0.05	0.89±0.01[d]	1.08±0.13[d]	1.26±0.16[d]	1.51±0.01[d]

Values are means±SD; [a]$P<0.05$ HFSC diet vs. control diet group; [b]$P<0.01$ HFSC diet vs. control diet group; [c]$P<0.05$ HFSC diet+NO-1886 vs HFSC diet group, [d]$P<0.01$ HFSC diet+NO-1886 vs HFSC diet group.

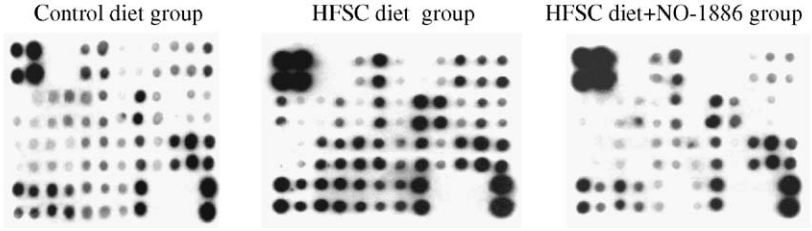

Fig. 4. Detection of cytokines in fat tissue samples on membrane antibody arrays. By comparing the signal intensities, relative expression levels of cytokines can be detected. The intensities of signals can be quantified by densitometry. Positive control can be used to normalize the results from different membranes for comparison. Each cytokine is represented by duplicate spots in the specific locations. Among these, A1 and A2, B1 and B2, HRP-conjugated antibody (positive control); C1 and C2, D1 and D2, NEG (negative control group); E1 and E2, EOTAXIN; F1 and F2, EOTAXIN-2; G1 and G2, G-CSF; H1 and H2, GM-CSF; I1 and I2, ICAM; J1 and J2, IFN-γ; K1 and K2, I-309; L1 and L2, IL-1α; A3 and A4, IL-1β; B3 and B4, IL-2; C3 and C4, IL-3; D3 and D4, IL-4; E3 and E4, IL-6; F3 and F4, IL-6SR; G3 and G4, IL-7; H3 and H4, IL-8; I3 and I4, IL-10; J3 and J4, IL-11; K3 and K4, IL-12P40; L3 and L4, IL-12P70; A5 and A6, IL-13; B5 and B6, IL-15; C5 and C6, IL-16; D5 and D6, IL-17; E5 and E6, IP-10; F5 and F6, MCP-1; G5 and G6, MCP-2; H5 and H6, M-CSF; I5 and I6, MIG; J5 and J6, MIP-1α; K5 and K6, MIP-1β; L5 and L6, MIP-1δ; A7 and A8, RNNTES; B7 and B8, TGF-β; C7 and C8, TNF-α; D7 and D8, TNF-β; E7 and E8, ATNFRI; F7 and F8, ATNFR II; G7 and G8, PDGF-BB; H7 and H8, TMP-2; I7 and I8, J7 and J8, BLANK; K7 and K8, NEG (Tris-buffered saline, negative control); L7 and L8, POS (HRP-conjugated antibody, positive control). Average net light intensity for each pair of cytokine spots was detected on the basis of gray-scale levels using LabWorks software.

62.20% (sTNF RII), and 269.13% (PDGF-BB) compared with that in the control diet group. Among these cytokines, ICAM, IL-1α, IL-1β, IL-6SR, MCP-1, MCP-2, TNF-α, TNF-β, and others showed the most predominant effects on the development of diabetes mellitus and atherosclerosis. On the contrary, almost 29 kinds of expressions of inflammation cytokines markedly decreased in the animals that received HFSC diet+NO-1886, and especially significant reductions were observed in the following inflammation cytokines; reduced by 98.58% (ICAM), 87.00% (INF-γ), 71.10% (IL-1α), 74.50% (IL-1β), 55.50% (IL-6SR), 94.01% (IL-11), 88.18% (IL-12P40), 100% (IL-12P70), 89.29% (IL-13), 72.60% (IL-16), 96.65% (IL-17), 72.26% (IP-10), 71.06% (MCP-1), 52.89% (MCP-2), 88.27% (MIG), 56.47% (TNF-β), 82.05% (sTNFRI), 99.56% (sTNFRII), and 84.61% (PDGF-BB) compared with that in the HFSC diet group.

3.5. Effect of NO-1886 on glucose tolerance, insulin response to glucose loading, and insulin resistance

Glucose tolerance is a function of glucose-stimulated insulin secretion, hepatic glucose output, and tissue insulin sensitivity. Both basal plasma glucose and insulin levels were higher in the HFSC diet group than in either the control diet or the NO-1886 supplementation groups. The NO-1886 supplementation and control diet groups showed a higher acute elevation in serum insulin in response to the oral glucose load at 30 min compared with the HFSC diet groups. The animals in the HFSC diet group showed marked glucose intolerance compared with those in both the control diet and HFSC diet+NO-1886 groups (Fig. 5A). The insufficient glucose removal observed in the HFSC diet group may have been caused by impairment of acute insulin secretion (absence of the first phase of insulin secretion) in response to the glucose load (Fig. 5B). The contribution of insulin sensitivity was explored by evaluating the clearance of plasma glucose as a function of time after insulin injection. This measure of whole body insulin sensitivity can be conveniently expressed as a Ki. The animals were injected i.p. with insulin and Ki values were determined (Fig. 5C). Since Ki reflects the rate of glucose removal, higher values indicate greater tissue insulin sensitivity. Insulin sensitivity was nearly 2-fold greater in the control diet and HFSC diet+NO-1886 groups compared with the HFSC diet group ($P<0.001$). Plasma glucose levels were significantly higher in the HFSC diet group at each time point, indicating that the HFSC diet group was in a severe insulin resistant state and that NO-1886 increased insulin sensitivity in the HFSC diet +NO-1886 group.

3.6. Pathological changes in aortas

The arch portions of the aortas were prone to develop fatty streak lesions in the HFSC diet group. Relative aortic fatty streak lesion area (percentage of whole area) was 41.8±9.52% for the HFSC diet group and 13.7±3.06% for the NO-1886 supplementation group, but no fatty streak was observed in the aortas in the control diet group (Fig. 6, the left sections). Tissue sections (5 μm) were cut from the aortic fatty streak lesions in the three groups and stained with oil red O (the middle sections) and haematoxylin-eosin (the right sections). Microscopic (×200 magnification) observation of the HFSC diet group clearly showed that the intima became rougher and thicker (black arrowhead showed). A lot of lipoid foam cells migrated to regions of intima (blue arrowhead showed), which associated with the injuries of internal elastic lamina. However, the microstructures of the aortas in the NO-1886 supplementation group only showed that intima became in some sort thicken, indicating that administration of NO-1886 significantly ameliorated the lesions. No lesions were observed in the control diet group.

4. Discussion

In the present study, we studied whether NO-1886, a lipoprotein lipase activator, has a beneficial effect on inflammation cytokines in miniature pigs with diabetes mellitus and atherosclerosis induced by a HFSC diet feeding. Our results showed that HFSC diet markedly increased levels of most of the inflammation cytokines expression of abdominal subcutaneous

Table 3
Cytokine release profiles in miniature pigs fed by different diet [a]

Inflammation factors [b]	Control diet	HFSC diet	HFSC diet +NO-1886	HFSC diet– control diet [c]/ control diet	HFSC diet +NO-1886/ HFSC diet
	(standardization)			(%)	(%)
EOTAXIN	0.4117	0.1829	0.4236	−90.56%	+131.60%
EOTAXIN-2	0.4484	0.6848	0.6732	+52.72%	NC
GCSF	0.0194	0.0425	0.0354	+119.00%	NC
GM-CSF	0.0116	0.0015	0.0001	−87.21%	−96.40%
ICAM-1	0.0498	0.0968	0.0014	+95.00%	−98.58%
IFN-gamma	0.1885	0.4380	0.0565	+132.00%	−87.00%
I-309	0.2234	0.2922	0.1588	+30.80%	−45.65%
IL-1 alpha	0.1860	0.3744	0.1081	+102.00%	−71.10%
IL-1 beta	0.0413	0.3253	0.0828	+687.00%	−74.50%
IL-6Sr	0.2223	0.4842	0.2154	+117.76%	−55.50%
IL-8	0.6056	0.8350	0.6444	+37.80%	−22.80%
IL-11	0.0026	0.1656	0.0099	+6385.66%	−94.01%
IL-12 p40	0.0858	0.3181	0.0376	+270.88%	−88.18%
IL-12 p70	0.0724	0.1438	0.0000	+98.55%	−100%
IL-13	0.0205	0.0026	0.0003	+87.25%	−89.29%
IL-15	0.1476	0.0353	0.0217	+76.08%	−38.42%
IL-16	0.1550	0.4280	0.1172	+176.96%	−72.60%
IL-17	0.0734	0.4943	0.0165	+574.00%	−96.65%
IP-10	0.2299	0.6276	0.1741	+172.89%	−72.26%
MCP-1	0.2461	0.5200	0.1505	+111.28%	−71.06%
MCP-2	0.1210	0.3734	0.1759	+209.00%	−52.89%
M-CSF	0.4170	0.6674	0.4168	+60.04%	−37.55%
MIG	0.0947	0.1746	0.0205	+84.30%	−88.27%
MIP-1 delta	0.4061	0.5688	0.5105	+40.05%	−10.25%
RANTES	0.4897	0.8164	0.6252	+66.73%	−23.40%
TGF-beta	0.3178	0.4681	0.2469	+47.31%	−47.25%
TNF-alpha	0.5433	0.6828	0.4963	+25.67%	−27.30%
TNF-beta	0.5300	0.7056	0.3070	+33.10%	−56.77%
sTNF RI	0.3613	0.5745	0.1031	+58.99%	−82.05%
sTNF RII	0.2501	0.4058	0.0018	+62.20%	−99.56%
PDGF-BB	0.1782	0.5470	0.0842	+269.13%	−84.61%
TIMP-2	0.7812	0.8760	0.6230	+12.14%	−29.12%

[a] Control diet group; HFSC diet, high-fat/high-sucrose/high-cholesterol diet group; HFSC diet+NO-1886, high-fat/high-sucrose/high-cholesterol diet supplement NO-1886 group.

[b] G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule-1; IFN-γ, gamma interferon; MCP-1, monocyte chemoattractant protein-1; M-CSF, granulocyte macrophage-colony stimulating factor; MIG, gamma interferon-induced monokine; MIP-1alpha, Macrophage Inflammatory Protein-1 alpha; TGF-β1, transforming growth factor β1; TNF-α, tumor necrosis factor-α; PDGF, platelet-derived growth factor; TIMP, tissue Inhibitor of metalloproteinases.

[c] "+" symbol was used to indicate an increase, "−" symbol was used to indicate a decrease, the values indicate the fold increase or decrease. NC means no change (less than or equal to fold difference from the level in the control diet group or the HFSC diet group).

adipose tissues. However, administration of NO-1886 significantly decreased the most inflammation cytokines expression.

Increasing evidence indicates that adipose tissue is an important source of cytokines (Ahima and Flier, 2000) and that adiposity contributes to a pro-inflammatory milieu (Nicklas et al., 2005; Yudkin et al., 1999; Wisse, 2004; Lyon et al., 2003), probably because that obesity alters adipose tissue metabolic and endocrine function and leads to an increased release pro-inflammatory factors such as TNF-á, interleukin-6 (IL-6), plasminogen activator inhibitor-1 and other cytokines (Ahima and Flier, 2000; Yudkin et al., 1999; Lyon et al., 2003; Vozarova et al., 2001; Dandona et al., 1998) that contribute to vascular inflammation and systemic inflammation, i.e. promoting insulin resistance (Kusunoki et al., 2000; Finking and Hanke, 1997), increased synthesis of acute-phase reactants in the liver [such as C-reactive protein, a marker for inflammatory reactions (Ryan and Nicklas, 2004; Lemieux et al., 2001; Forouhil et al., 2001; Saijo et al., 2004; Yudkin et al., 1999; Heinrich et al., 1995)] and a risk factor for the development of type 2 diabetes (Nakanishi et al., 2003), or activation of macrophages in atheromatous plaques (Vozarova et al., 2002).

Several evidences have shown that long-term imbalance between intake and expenditure of fat is a central factor in the aetiology of obesity (Marfella et al., 2004; Flatt, 1995). Our current food supply is high in fat, sucrose and cholesterol. We hypothesized that HFSC diet promoted obesity by increasing energy intake, thus increasing the probability of positive energy balance and weight gain, ultimately induced to obesity. In this study, all the animals were given the same amount of diet.

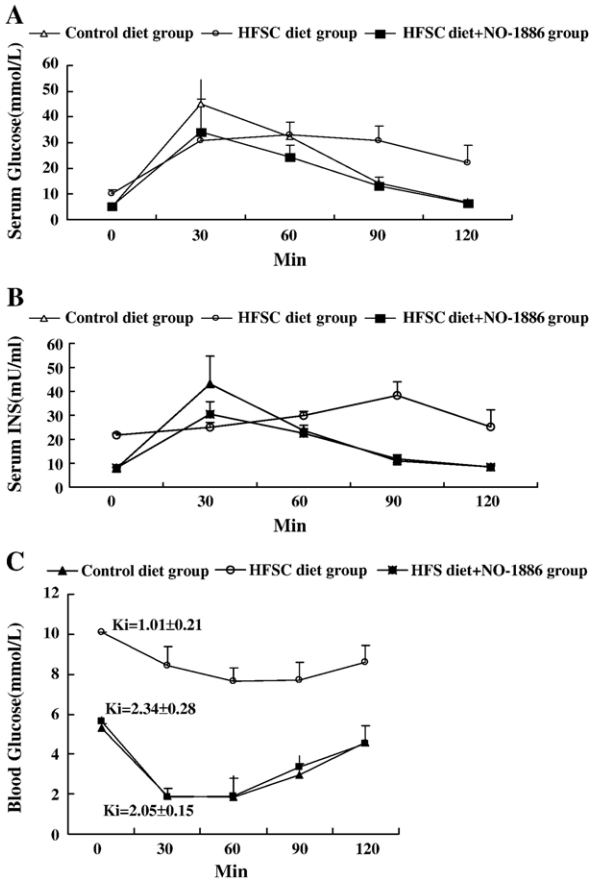

Fig. 5. Effects of control diet or HFSC diet with and without NO-1886 (1.0%) for 5 months on the levels of glucose and insulin in response to oral glucose loading, blood glucose (A) and serum insulin (B) in an oral glucose tolerance test. The whole-body insulin sensitivity was expressed as a Ki. Higher Ki values indicate greater tissue insulin sensitivity. Insulin sensitivity was nearly 2-fold greater in the control diet and HFSC diet+NO-1886 groups compared with the HFSC diet group ($P<0.001$) (C). $n=5$ per group. Each point represents the mean±SD. $n=5$ per group.

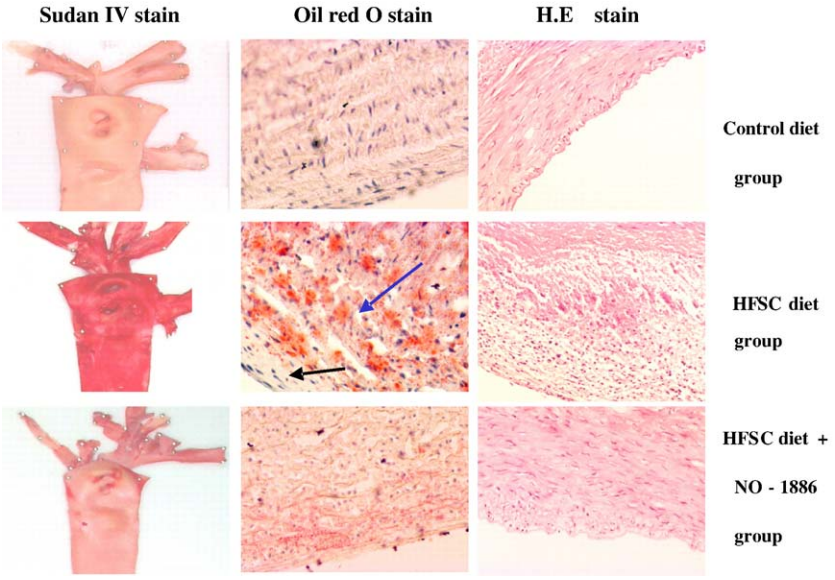

Fig. 6. NO-1886 inhibited the atherosclerosis lesions induced by HFSC diet in Chinese Bam-miniature pigs. The left representative photographs of pinned-out aorta from control diet pigs and HFSC diet-fed pigs and HFSC diet + NO-1886-fed pigs. The middle sections stained with Oil red O (×200 magnification). The right sections (5 mm thick) were taken at the aortic arch from each pig and stained with haematoxylin-eosin (×200 magnification).

During our experiment, they had good appetites and dejectas were normal. The results show that feeding of HFSC diet to miniature pigs for 5 months were induced to obesity, especially visceral obesity, related metabolic syndrome such as hypertriglyceridemia, impaired glucose tolerance, insulin resistance and pro-inflammatory cytokines expressions. Administration of NO-1886 reduced body weight and visceral fat mass and improved metabolic syndrome. The most significant finding in the study was the reduction by NO-1886 in almost 29 different levels of inflammation-associated cytokines.

The mechanisms by which NO-1886 decreases the expressions of inflammation cytokines and alleviates inflammation status remain unclear.

Some time ago, Eckel RH suggested that there was a role for tissue-specific regulation of lipoprotein lipase in treatment of obesity (Eckel, 1989). Lipoprotein lipase is a key enzyme that hydrolyses triglycerides (which is an important carrier of blood lipid) into free fatty acids and glycerol. The free fatty acids can be used for metabolic fuel in tissues such as muscle. Jensen DR found that diet-induced obesity in mice can be prevented by creating transgenics overexpressing a skeletal muscle-specific human lipoprotein lipase gene (Jensen et al., 1997). Tomonari et al. (2004) also reported that overexpression of lipoprotein lipase in transgenic watanabe heritable hyperlipidemic rabbits improves hyperlipidemia and obesity.

The compound NO-1886 has previously been reported to increase post-heparin plasma lipoprotein lipase activity (Tsutsumi et al., 1996; Hagi et al., 1997). Hara et al. examined rats fed with fructose and treated with NO-1886 for 1 month. They found that administration of NO-1886 group had a lower 24 mean respiratory quotient (RQ) than control group (Hara et al., 1998). Recently, Kano and Doi administered NO-1886 to the experimental ovariectomized (OVX) animal model from 5 to 13 weeks after the operation. Then, it was found that NO-1886 decreased accumulation of visceral fat, suppressed the increase in body weight, decreased the respiratory quotient by increasing expression of the fatty acid oxidation-related enzymes and UCP3, which are related to fatty acid transfer and fat use (Kano and Doi, 2006). Kusunoki et al. (2005) found the same effects of NO-1886's antiobesity in rats. They also reported that NO-1886 increased fat oxidation (indexed by lowered RQ) and metabolic rate, inhibited fat accumulation, and reduced insulin resistance in high-fat-induced obesity and type 2 diabetes animal model rats (Kusunoki et al., 2000). Therefore, it is considered that increasing lipoprotein lipase activity probably leads to reduced rate of weight gain through greater disposal of ingested fats as metabolic fuel in peripheral tissues. In our previous studies, NO-1886 decreased ectopic lipid deposition (Yin et al., 2004a,b) and inhibited size of adipocytes (Yin et al., 2004a,b). In this paper, we found that NO-1886 supplementation caused a marked increase in postprandial post-heparin plasma lipoprotein lipase activity (Fig. 3). Furthermore, NO-1886 inhibited body weight gain and decreased abdominal fat accumulation by 42.23% of miniature pigs fed by HFSC diet (Fig. 2). These results are consistent with the previous observation. Not surprisingly, approaches that reducing ectopic lipid deposition and inhibiting weight gain will improve pro-inflammatory cytokines levels and reduce the severity of their resultant pathologies.

In summary, administration of a novel compound NO-1886 to HFSC diet-fed miniature pigs significantly decreased the levels of plasma C-reactive protein and plasminogen activator inhibitor-1 and the expression of most pro-inflammation cytokines in fat tissues, alleviated inflammation status. These effects may account for the role of NO-1886 in improving atherosclerosis and diabetes mellitus. NO-1886 is potentially beneficial for the treatment of inflammation status, reduced the severity of the resultant atherosclerosis and diabetes mellitus.

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