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Regulation of gut-derived resistin-like molecule β expression by nutrients

Junko Fujio [a,b], *Akifumi Kushiyama* [b], *Hideyuki Sakoda* [b], *Midori Fujishiro* [b], *Takehide Ogihara* [c], *Yasushi Fukushima* [a], *Motonobu Anai* [d], *Nanao Horike* [a], *Hideaki Kamata* [e], *Yasunobu Uchijima* [a], *Hiroki Kurihara* [a], *Tomoichiro Asano* [a,e,*]

[a] *Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

[b] *Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

[c] *Division of Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research on Human Diseases, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Sendai, Japan*

[d] *Institute for Adult Diseases, Asahi Life Foundation, 1-6-1 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan*

[e] *Department of Medical Science, Graduate School of Medicine, University of Hiroshima, 1-2-3 Kasumi, Minami-ku, Hiroshima City, Hiroshima 734-8551, Japan*

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ABSTRACT

Resistin was initially identified as a protein, secreted by adipocytes, which inhibits insulin action and adipose differentiation. The three proteins homologous to resistin were identified and given the names resistin-like molecules (RELM) α, β and γ. Resistin and RELMα are abundantly expressed in adipose, but RELMβ and RELMγ are secreted mainly from the gut. Since nutrient composition greatly affects insulin sensitivity, we investigated the regulatory effects of various nutritional factors in food on the expressions of resistin family proteins.

First, mice were given diets with different nutritional compositions (high-carbohydrate, high-protein and high-fat) for 2 weeks. RELMβ mRNA expression in the intestines was markedly suppressed by the high-protein and high-carbohydrate diets, while slightly but not significantly upregulated by the high-fat diet. In the epididymal fat, resistin expression was unchanged, while RELMα expression was markedly decreased by the high-carbohydrate diet. Taking into consideration that humans have neither RELMα nor RELMγ, our subsequent studies focused on RELMβ expression. We used the human colon cancer cell line LS174T. Treatments with insulin and TNFα as well as stearic acid, a saturated free fatty acid, upregulated RELMβ expression, while D-glucose downregulated RELMβ. These results suggest RELMβ expression to be regulated directly by nutrients such as glucose and saturated free fatty acids including stearic acid, as well as by hormones including insulin and TNFα. These regulations may play an important role in the nutrient-associated induction of insulin resistance.

* *Corresponding author*. Tel.: +81 82 257 5135; fax: +81 82 257 5136.
E-mail address: asano-tky@umin.ac.jp (T. Asano).

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1. Introduction

Resistin and its related proteins, *i.e.* resistin-like molecules (RELMs) α, β and γ, are a family of recently identified proteins [1,2]. They share an N-terminal signal sequence and a C-terminal region with a unique structure that contains 10 cysteine residues [3]. Resistin was identified as an adipocyte secreted factor, expression of which is increased in genetically obese (*ob/ob* and *db/db*) mice [4]. Furthermore, administration of resistin reportedly impairs glucose tolerance and reduces insulin action in normal mice, both of which are reversed by immunoneutralization with anti-resistin antibody [4]. Resistin knock-out mice were also described as having lower fasting blood glucose [5]. However, there are conflicting observations regarding its function as a factor responsible for insulin resistance [6–9].

RELMα is a secreted protein of 111 amino acids that has been identified in rats and mice and is expressed in the lungs, white adipose tissue and the intestines. There is a difference between the two species in that RELMα expression in white adipose tissue is much lower in rats than in mice [2,3]. This protein has been shown to inhibit the differentiation of adipocytes *in vitro* [10]. RELMα is induced by Th2 type cytokines in rodent pulmonary epithelial cells, and thus is likely to be involved in the inflammatory response [11]. RELMγ was also initially identified in the nasal respiratory epithelium of rats [2], and was revealed to be expressed in bone marrow, peripheral blood granulocytes, the spleen, lungs and pancreas as well as the large and small intestines of mice [2,12,13].

RELMβ is highly expressed in goblet cells of the murine colon and secreted in response to bacterial colonization [14], and thus was suggested to play an important role in defense against nematode parasitization in mice [15]. On the other hand, we previously reported that RELMβ and RELMγ are present in blood, and that their serum concentrations and expressions in the colon were elevated in insulin resistant models such as obese *db/db* mice and high-fat-fed mice [16]. In addition, transgenic mice which overexpressed RELMβ in the liver, exhibited hyperglycemia, hyperlipidemia and fatty liver [17]. Thus, we consider intestine-derived RELMβ to be involved in insulin resistance.

The first objective of this study was to investigate the regulatory effects of nutritional factors in different diets on the expressions of resistin and RELMs. Interestingly, the expression of RELMβ, but not resistin, was found to be strongly influenced by different dietary compositions. Although there are four genes encoding this protein family in the mouse, only resistin and RELMβ have been identified in the human genome sequence [2]. Thus, we focused on the regulation of RELMβ expression, and performed additional experiments using cultured cells to examine whether nutritional factors, as well as hormones such as insulin and TNFα, are direct regulators of RELMβ expression. Herein, we show the regulation of gut-derived RELMβ to be regulated by both nutrients and hormones, and that its upregulation may be involved in the pathogenesis of diet-derived insulin resistance.

2. Materials and methods

2.1. Reagents and antibodies

All reagents were of analytical grade and anti-RELMβ antibody was purified as previously described [17].

2.2. Animal studies

Six-week-old mice (C57BL/6J) were purchased from CLEA Inc and housed under conventional conditions. All animal studies were performed after 2–3 days acclimation period and mice were anesthetized with pentobarbital. To determine RELMβ expression levels in fed and fasted states, the colon was excised from both mice fed *ad libitum* and those fasted for 18 h ($n = 3$ per group). In the fasted state, both the colon and the ileum were collected to assess the correlation between RELMβ mRNA levels in these tissues ($n = 22$). In the dietary studies, animals were divided into four groups receiving different diets, *i.e.* high-carbohydrate (CA), high-protein (P), high-fat (HF) and control (C) diets, and were fed *ad libitum* for 2 weeks ($n = 4$–5 per group) or fed once ($n = 6$ per group), to assess both acute and chronic effects of these diets. The compositions of the diets are shown in Table 1. With 2 week feeding, at the end of the 2-week period, the animals were fasted for 18 h. Then, blood, colon and epididymal fat, as a representative white adipose tissue, samples were collected. Tissue samples were homogenized in an adequate amount of ice-cold Isogen (Nippon Gene) directly for mRNA extraction or ice-cold Lysis Buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Triton-X 100, 2 mM PMSF, 2 μg/ml aprotinin, 5 μg/ml leupeptin) after careful removal of stool, for Western blotting. Serum was separated, after a sufficient time at room temperature to allow coagulation, by centrifugation at 3000 rpm for 20 min followed by 1 min at 15000 rpm. Lipid and other parameters were measured in the sera obtained.

Animal care and procedures for the experiments were performed according to the Japanese guidelines for the care and use of experimental animals.

Table 1 – Dietary compositions

	Control diet (3.58 Kcal/g)		High carbohydrate (3.55 kcal/g)		High protein diet (3.47 kcal/g)		High fat diet (6.66 kcal/g)	
	%weight	%kcal	%weight	%kcal	%weight	%kcal	%weight	%kcal
Protein	23.3	26	13	14	70	79.8	24.2	14.6
Fat	5.3	13.3	1	4.4	1	5.7	60	81
Carbohydrate	53.8	60.1	80	81.6	10	12.5	7.3	4.4

2.3. Intraperitoneal glucose tolerance tests

Glucose tolerance tests were performed after the 2-week feeding period. After an overnight fast, 2 g/kg D-glucose was injected intraperitoneally after the initial glucose measurement. Glucose levels were again determined at 15, 30, 60, 90 and 120 min after the injection. Glucose was measured by tail snipping. Three or four mice from each group were subjected to this test.

2.4. Cell culture

LS174T cells were obtained from the Cell Resource Center for Biomedical Research (Sendai, Japan), and cultured in RPMI 1640 (Sigma) medium supplemented with 10% FCS (Invitrogen), Penicillin 100 U/ml and Streptomycin 100 μg/ml (GIBCO Invitrogen) at 37 °C in 5% CO_2. Cells were cultured on 24 well plates (IWAKI) for the extraction of mRNA for stimulation tests. At 80% confluence, each well was washed twice with PBS and subsequently incubated under various conditions described below for 24 h, and the cells were then subjected to the mRNA extraction.

For insulin and TNFα stimulation, insulin and TNFα were added to RPMI 1640 to give final concentrations of 100 nM and 100 ng/ml, respectively. For glucose stimulation, RPMI 1640 supplemented with D-glucose to achieve final concentrations of 5, 11 or 25 mM was used and RPMI 1640 containing L-glucose at the same concentrations was used for the controls. Furthermore, linoleic acid (LA), oleic acid (OA) and stearic acid (SA) resolved in ethanol and conjugated with 20% bovine serum albumin (BSA) were added to RPMI 1640 to give two final concentrations, 0.5 and 2.0 mM, for each FFA. For control samples, medium adjusted only with ethanol and BSA was used.

2.5. RNA isolation and quantification by real time quantitative polymerase chain reaction

Total RNA was extracted from murine tissue samples, or cultured LS174T cells, using Isogen (Nippon Gene) according to the manufacturer's instructions. The cDNA was synthesized from total RNA using a First Strand cDNA Synthesis Kit for RT-PCR (Roche Diagnostics) according to the manufacturer's instructions. The oligonucleotide primers were designed using program Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/) and produced by Japan Bio Service, (Saitama, Japan). mRNA expressions for RELMs and resistin were quantified on a Light Cycler Instrument (Roche) using Light Cycler DNA Master SYBR Green I. The results were standardized against internal controls, m36B4 or h36B4 for the mouse tissue and LS174T cell-derived mRNA, respectively. The primer sequences used for human RELMβ (hRELMβ), mouse resistin (mResistin), mouse RELMβ (mRELMβ), mouse RELMγ (mRELMγ), m36B4 and h36B4 are shown in Table 2. The primers for hRELMβ and h36B4 were used as described previously [14,18].

2.6. Histological analysis

Colonic tissues were routinely embedded in paraffin; approximately 5 μm-thick slices were obtained from these samples. Slices were stained with hematoxylin and eosin (HE) to compare the number of goblet cells. Immunostaining was performed according to the microwave antigen-retrieval technique, using purified anti-mRELMβ antibody (1:500) and a VECSTATIN ABC kit (Vector labs), following the manufacturer's instructions.

2.7. Western blotting

Twenty micrograms of protein extracted from homogenized colonic tissue or 4 μl of serum was boiled in Laemmli sample buffer containing 100 mmol/l dithiothreitol. Samples were subjected to SDS-PAGE, transferred to Hybond-P membranes (GE Healthcare, Bioscience Inc.), and immunoblotted using purified anti-mRELMβ antibody (1:1000). Proteins were visualized with enhanced chemiluminescence (ECL) and exposed to ECL film (GE Healthcare, Bioscience Inc.). The band intensity was analyzed as described previously [16].

2.8. Statistical analysis

Stat View-J 5.0 software for windows (SAS Institute Inc.) was used for statistical analysis. Results are expressed as mean ± S.E. In the multiple comparisons, ANOVA followed by the post hoc Fisher's PLSD test was used to compare means between pairs of groups. The unpaired *t*-test was also used to compare means between pairs of groups.

3. Results

3.1. Characterizations of feeding groups, energy intake and changes in serum lipid, glucose and insulin levels

The body weights, epididymal fat weights, glucose levels, insulin levels and serum lipid levels at the start and after 2

Table 2 – Primers used for real-time PCR

	Sense	Antisense
m-Resistin	TCATTTCCCCTCCTTTTCCT	AAGCGACCTGCAGCTTACA
m-RELMα	TCCAGCTAACTATCCCTCCACTGT	CAGTAGCAGTCATCCCAGCA
m-RELMβ	CAAAAAGCTAGAACTGAGCTCCAG	TAGTAATATGAAGACAATGAGTCAGG
m-RELMγ	CTTGCCAATCGAGATGACTG	TTTCCAAGTTGGGATTGTGC
m-36B4	GCTCCAAGCAGATGCAGCA	CCGGATGTGAGGCAGCAG
h RELMβ	CACCCAGGAGCTCAGAGATCTAA	ACGGCCCCATCCTGTACA
h-36B4	CCACGCTGCTGAACATGCT	TCGAACACCTGCTGGATGAC

Table 3a – Characteristics of the dietary groups of the study

	Control	High carbohydrate	High protein	High fat
Body weight (g)	19.2 ± 0.32	19.2 ± 0.15	19.4 ± 0.33	19.2 ± 0.22
Blood glucose (mg/dl)	66.4 ± 3.24	67.8 ± 6.34	64.7 ± 4.21	57.9 ± 3.03
Insulin (ng/ml)	2.91 ± 0.09	3.20 ± 0.31	2.80 ± 0.50	3.72 ± 0.17
Triglyceride (mg/dl)	73.8 ± 5.32	69.6 ± 4.47	76.3 ± 6.56	65.9 ± 3.40
Cholesterol (mg/dl)	82.6 ± 3.07	61.8 ± 6.51	84.6 ± 4.79	80.5 ± 7.27
NEFA (μEq/l)	1.33 ± 0.06	1.28 ± 0.18	1.14 ± 0.07	1.00 ± 0.05

Values are indicated as mean ± S.E. (n = 4–6).
No statistically significant differences between the feeding groups were observed.

Table 3b – Characteristics of the dietary groups at the end of the 2-week feeding period

	Control	High carbohydrate	High protein	High fat
Body weight (g)	21.1 ± 0.6	20.4 ± 0.6	19.7 ± 1.0	23.0 ± 0.3
Blood glucose (mg/dl)	84.6 ± 6.0	90.0 ± 8.4	120.6 ± 21.7	125.4 ± 17.0
Insulin (ng/ml)	5.82 ± 1.16	4.47 ± 0.66	4.64 ± 2.70	14.50 ± 3.43*
Triglyceride (mg/dl)	60.7 ± 4.8	49.5 ± 4.6	45.2 ± 9.8	61.2 ± 4.1
Cholesterol (mg/dl)	87.4 ± 6.0	108.1 ± 5.0*	46.2 ± 6.4*	98.3 ± 5.3
NEFA (μEq/l)	0.92 ± 0.09	0.86 ± 0.01	0.94 ± 0.10	0.92 ± 0.04
Epididymal fat (mg)	115.8 ± 16.1	49.6 ± 2.6*	129.1 ± 29.9	404.6 ± 17.0*

n = 4–6.
* $p < 0.05$.

weeks of feeding are shown in Tables 3a and 3b, respectively. Body weights, glucose levels and lipid profiles at the beginning of the feeding period did not differ significantly among the groups (Table 3a). Body weights of the three different dietary groups did not differ significantly from that of the control group at the end of the 2-week feeding period, though the high-fat group tended to be heavier (Table 3b). Furthermore, the epididymal fat mass of the high-fat group was significantly larger than that of the control group at the end of the 2-week feeding period (C 115.8 ± 16.1 mg, HF 404.6 ± 17.0 mg; $p < 0.01$) (Table 3b). The high-carbohydrate group had a significantly reduced adipose tissue mass as compared to the control group (C 115.8 ± 16.1 mg, CA 49.6 ± 2.6 mg; $p < 0.05$) (Table 3b).

In contrast to the adipose depot mass, serum total cholesterol was slightly elevated in the high-carbohydrate group by the second week (C 87.4 ± 6.0 mg/dl, CA 108.1 ± 5.0 mg/dl; $p = 0.02$). The high-protein group, however, had significantly lower levels at the end of the second week (C 87.4 ± 6.0 mg/dl, P 46.2 ± 6.4 mg/dl; $p < 0.01$) (Table 3b). Serum non-esterified fatty acids (NEFA) and triglyceride levels did not differ significantly among the groups.

3.2. *Impaired glucose tolerance in the high-fat diet group*

To assess whether these diets impair glucose tolerance, intraperitoneal glucose tolerance tests were performed at the end of the 2-week feeding period, as described in Section 2 (Fig. 1). The high-fat group showed a significantly greater glucose rise than the control group, and showed this serum glucose elevation was sustained beyond the 120 min of the test. No such obvious glucose intolerance was detected in either the high-carbohydrate or the high-protein group.

3.3. *Expression levels of RELMβ and RELMγ in the colon, and of resistin and RELMα in white adipose tissue*

RELMβ expression profiles in fasted and fed states, and the correlations between levels in the colon and ileum are presented in Fig. 2. In the fasted state, the RELMβ protein level was downregulated 43.3 ± 17.2% as compared with that in the fed state (*ad libitum*), which suggests that the diet itself affects RELMβ expression (Fig. 2A and B). There was a positive correlation between RELMβ mRNA levels in the ileum and the colon ($r^2 = 0.604$, $p = 0.0002$), although the RELMβ mRNA level

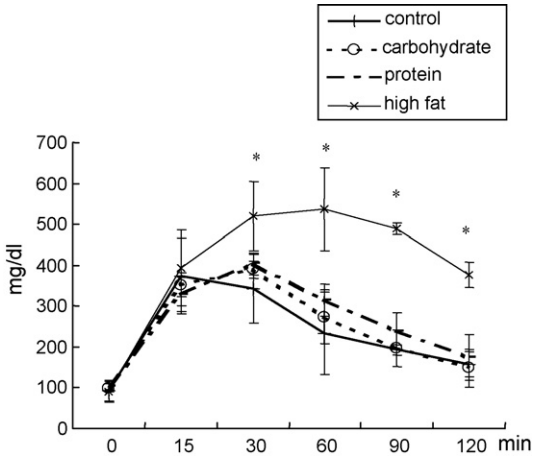

Fig. 1 – Impaired glucose tolerance in the high-fat diet group. The results of intra-peritoneal glucose tolerance tests done at the end of the second week are shown. Asterisks (*) denote glucose values significantly different from those of the control group. Bars indicate standard errors (n = 3–4).

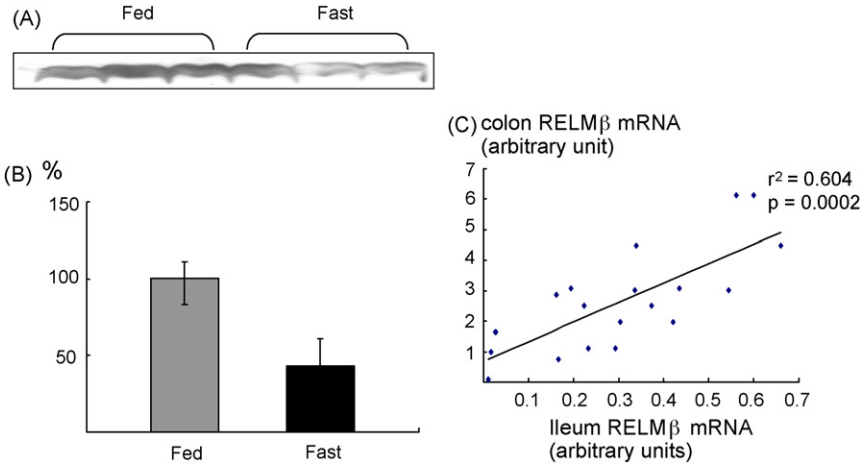

Fig. 2 – Altered expressions of RELMβ in fed and fasted states and comparison of RELMβ expressions in the ileum and colon. The protein level is shown as band (A), quantification as band (B), using NIH image. Asterisks (*) denote values in the state, which are significantly different from those in the fed state. Bars indicate standard errors ($n = 3$). The RELMβ mRNA level is shown in a scatter plot. The X-axis represents RELMβ from the ileum, the Y-axis that from the colon. Scales of the two are arbitrary but the values correspond to each other.

in the colon was more variable than that in the ileum, being up to 10 times higher (Fig. 2C). A positive correlation between RELMβ levels in serum and the colon was demonstrated previously [16].

As shown in Fig. 3A, interestingly, it was revealed that the high-carbohydrate and high-protein diets had markedly decreased RELMβ mRNA expression by the end of the 2-week feeding period (C 1.0 ± 0.34, CA 0.002 ± 0.001; $p = 0.02$, P 0.09 ± 0.06; $p < 0.01$) in the colon, while RELMβ mRNA expression in the high-fat group was slightly higher than that of the control group, but this difference was not statistically significant (Fig. 3A). In the ileum and serum, the same tendency was observed, as shown in Fig. 3B and C, although only serum RELMβ in the high protein group changed significantly (C 1.00 ± 0.15, CA 0.67 ± 0.33, P 0.46 ± 0.02, $p < 0.02$, F 1.31 ± 0.16 in serum, C 1.00 ± 0.24, CA 0.73 ± 0.10, P 0.68 ± 0.06, F 0.95 ± 0.23 in the ileum). These results suggest a strong influence of nutritional components on RELMβ expression in the colon. Furthermore, a single feeding produced no significant change in RELMβ mRNA (1.00 ± 0.13, CA 1.06 ± 0.23, P 0.84 ± 0.04 or F 0.82 ± 0.07) in the colon. The RELMβ mRNA level was changed by the diet itself, although repetitive and chronic stimulation was needed for those dietary components to change the RELMβ mRNA level.

The resistin mRNA analysis of white adipose tissue conducted during the second week of the feeding period, showed no significant differences among the groups (Fig. 4A). The RELMα mRNA expression levels in white adipose tissue

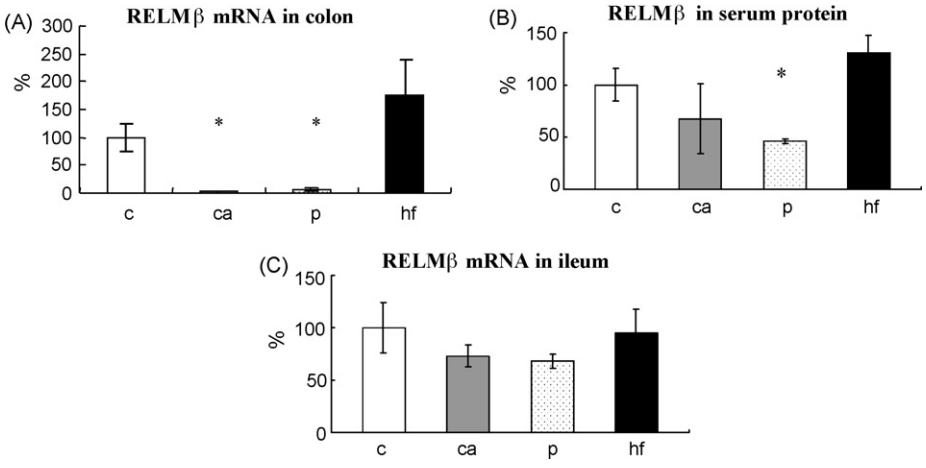

Fig. 3 – Altered expressions of RELMβ in the colon, ileum and serum in response to various dietary compositions. The mice were given a control, high-carbohydrate, high-protein or high-fat diet for 2 weeks. RELMβ expressions in the colon (A), serum (B) and ileum (C) were investigated and the data are presented as percentages of the control group values. Asterisks (*) denote values significantly different from those of the control group. Bars indicate standard errors ($n = 4$–6).

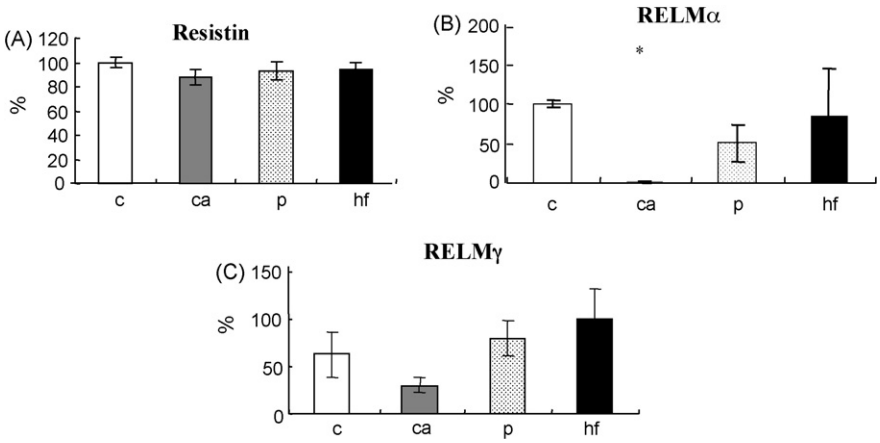

Fig. 4 – Expressions of resistin and RELMα mRNAs in adipose tissue and RELMγ in the colon in response to various dietary compositions. The mice were given a control, high-carbohydrate, high-protein or high-fat diet for 2 weeks. The expressions of resistin (A) and RELMα (B) mRNAs in adipose tissue and RELMγ (C) in the colon were investigated and the data are presented as percentages of the control group values. Asterisks (*) denote values significantly different from those of the control group. Bars indicate standard errors (*n* = 4–6).

are presented in Fig. 4B. RELMγ mRNA expressions in the colon did not differ among the dietary groups (Fig. 4C). Two-week feeding of a high-carbohydrate diet significantly suppressed RELMα expression as compared to the control group, while the high-protein and high-fat diets had no marked effects.

3.4. *Histological analysis*

Representative RELMβ immunohistochemistry of the colon, the major RELMβ production site, for each dietary group, is presented in Fig. 5. The high-carbohydrate and high-protein

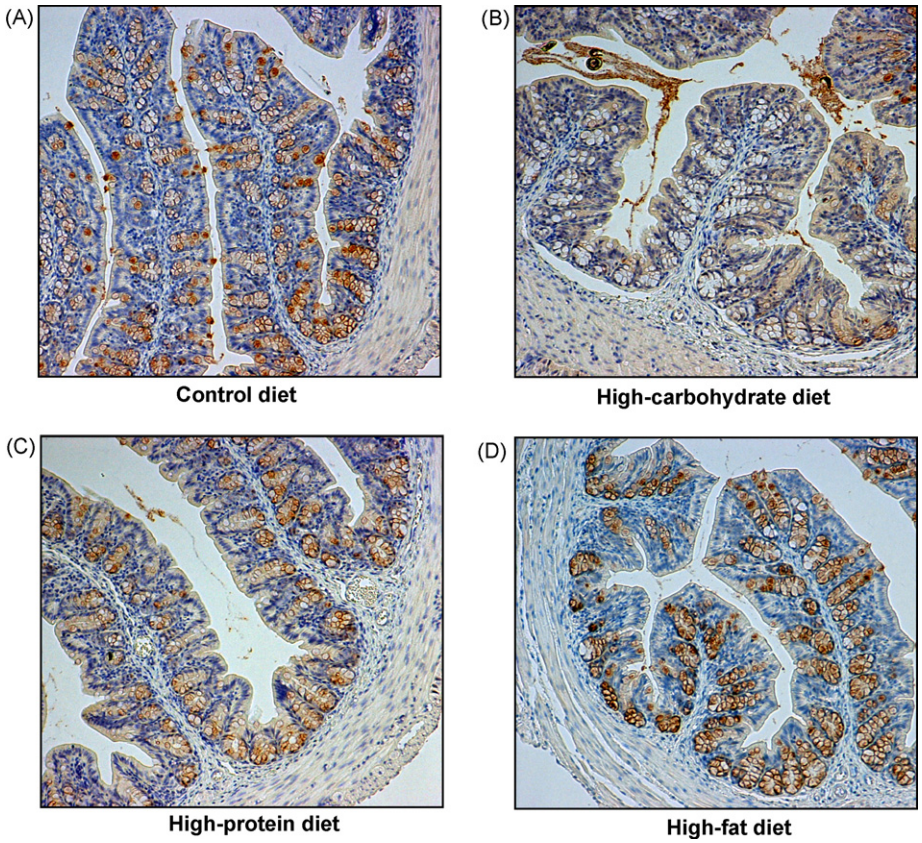

Fig. 5 – Colonic Immunohistochemistry of RELMβ. Colonic immunohistochemistry of RELMβ (magnification 100×) for each dietary group is shown. RELMβ is identifiable by its brown appearance. (A) Control diet, (B) high-carbohydrate diet, (C) high-protein diet, (D) high-fat diet.

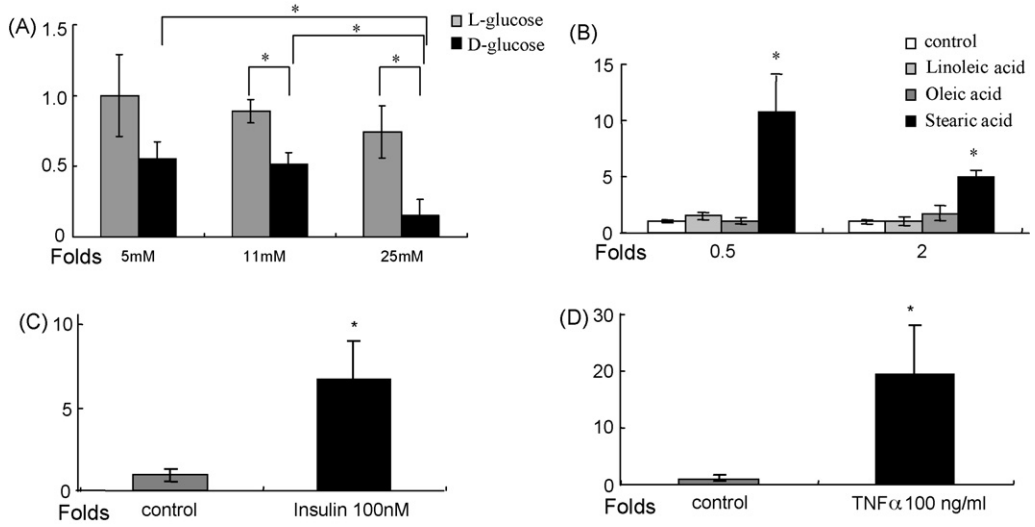

Fig. 6 – hRELMβ mRNA expression in LS174T cells with different stimuli. The hRELMβ mRNA expressions in LS174T cells after 24 h stimulation, with the agents shown at the indicated concentrations, are presented. (A) The cells were incubated with D-glucose or L-glucose at concentrations of 5 mM, 11 mM and 25 mM. The data are presented as the fold increase compared to the L-glucose group. (B) The cells were incubated with or without linoleic acid, oleic acid or stearic acid at concentrations of 0.5 and 2 mM. The data are shown as fold increases compared to the group without stimulation. (C) The cells were incubated with or without 100 nM insulin. (D) The cells were incubated with or without 100 ng/ml TNFα. Data from four separate experiments are presented and the bars indicate standard errors. The asterisks (*) denote values significantly different from those of the control group.

diet groups showed significantly less RELMβ expression than the control and high-fat diet groups. HE-stained preparations from all dietary groups were compared for the number of goblet cells. The absence of significant differences in numbers of intestinal goblet cells, among the groups, was also confirmed.

3.5. *Changes in hRELMβ mRNA expression in a human colon cancer cell line with various stimulations*

Expressions of hRELMβ mRNA in the human colon cancer cell line LS174T, were compared after stimulation with D-glucose or L-glucose, insulin, TNFα and three types of FFA, as described in Section 2. The results are presented in Fig. 6. Stimulation with D-glucose at 5, 11 and 25 mM significantly reduced the RELMβ mRNA expression in a concentration dependent manner (Fig. 6A). The three FFA exerted different effects on mRNA expression. Stearic acid stimulation resulted in marked upregulation of the RELMβ mRNA level (0.5 mM stearic acid, 10.7 ± 3.3 fold; $p < 0.01$, 2.0 mM stearic acid, 3.4 ± 0.38 fold; $p < 0.01$), while linoleic and oleic acids had no significant effects (Fig. 6B).

Stimulation with 100 nM insulin induced a 6.7-fold increase in mRNA expression ($p < 0.01$) (Fig. 6C). TNFα stimulation markedly increased RELMβ mRNA expression, by approximately 20 fold ($p < 0.01$) (Fig. 6D).

4. Discussion

One major factor contributing to Type 2 diabetes mellitus is insulin resistance, and obesity is known to be the most common factor inducing insulin resistance. Pathophysiological states (*i.e.* insulin resistance, obesity, and low-grade inflammation) are major and synergistic components of the metabolic syndrome. It was recently demonstrated that adipocytes are not only a lipid depot site, but also actively produce and secrete hormones and cytokines [19]. Resistin is one of these adipocyte-derived proteins and was suggested to play a role in the development of insulin resistance [4].

In addition, it was revealed that resistin and three structurally related RELMs constitute a resistin family [2,3,14]. Only RELMβ among these three RELMs is present in humans. Intestinal RELMβ secretion is reportedly increased in response to bacterial colonization [14] and has been suggested to be involved in the defense mechanism against nematode infestation in mice [15]. On the other hand, administration of RELMβ via the bloodstream induces acute hepatic insulin resistance [20], and transgenic mice over-expressing RELMβ in the liver were shown to exhibit hyperglycemia, hyperlipidemia and fatty liver [17]. These findings suggest that RELMβ is involved in both inflammatory responses intrinsic to the intestine and insulin resistance, particularly in the liver, and therefore may be an important link between these two pathophysiological states. Taking the aforementioned background factors into consideration, we carried out this study to investigate the regulatory effects of various nutritional factors in food on the expressions of RELMβ and other isoforms.

The effects of different nutritional components of diets are now receiving attention, especially in relation to obesity. With the intention of preventing and treating obesity and related diseases, intervention trials have been undertaken [21,22]. Diets rich in carbohydrate and low in fat have been employed, and have been found to reduce the incidence of diabetes by up

to 60%. Diets of similar composition are also recommended by medical societies for the treatment of diabetes [23,24]. Another study revealed that a high-protein diet resulted in substantial and sustained improvements in waist circumference, triglycerides and insulin, whereas with a high carbohydrate diet these changes were more modest [25]. In patients with Type 2 diabetes, a high protein diet reportedly improved glucose metabolism, due to the stimulatory effect of protein on insulin secretion [26].

In the present study, neither the high-carbohydrate nor the high-protein diet for 2 weeks induced either hyperinsulinemia or hyperglycemia in the fasting condition, nor was there any obvious glucose tolerance impairment in mice. Furthermore, epididymal fat tissue masses in both groups were reduced or were similar to those of the control group. These results are in a good accordance with the reported observations in a clinical trial [25]. In this study, we first demonstrated RELMβ expression in mice to be strongly influenced by whether the animals were fasted or fed, and differences in dietary nutritional composition, while resistin expression in adipose tissues did not differ significantly among the dietary groups. Resistin levels in white adipose tissue are reportedly higher in insulin resistant rodent models [4], though others have described contrasting observations [6–8]. Post-transcriptional and/or post-translational modifications, that consequently affect the secretion rate of the protein, have been suggested as possible explanations for this discrepancy.

Since RELMβ is the only RELM in humans, we focused on the regulation of colonic RELMβ expression, which was significantly suppressed in both the high-protein and the high-carbohydrate group. The histological investigations ruled out suppressed RELMβ expression due to a reduced number of goblet cells, and indicated that RELMβ secretion is markedly influenced by nutrients. Therefore, we speculate that protein and carbohydrate exert suppressive effects, or alternatively that an as yet unknown lipid, induces RELMβ expression. We also considered the possible involvement of insulin and TNFα, serum concentrations of which are increased in high-fat diet-induced insulin resistance. To examine these possibilities, a human colon cancer cell line, LS174T, which has been shown to express human RELMβ (RELMβ) under basal conditions [14], was subjected to various culture conditions. The initial incubation of these cells with D-glucose induced significantly lower RELMβ expression, in a D-glucose concentration dependent manner, than the same L-glucose concentrations. Subsequently, it was revealed that only the saturated FFA, *i.e.* stearic acid, had significant inducing effects on RELMβ mRNA expression, while the other two free fatty acids had little impact.

We also demonstrated insulin and TNFα to markedly increase RELMβ expression. Induction of RELMβ expression by TNFα is an observation in good accordance with previous study results showing induction of RELMβ expression by Th2 cytokines such as Il-4 and IL-13 [15]. Furthermore, the presence of several STAT6 and NFκB elements in the promoter region of human RELMβ was disclosed by sequence analysis [14].

Taking these results together, we can suggest possible mechanisms underlying diet-induced RELMβ regulation. First, repetitive and chronic stimulation by certain free fatty acids, such as stearic acid, or glucose, *per se*, increased and decreased RELMβ expression, respectively. Second, although no supporting data were obtained in this study, it is reasonable to speculate that the different nutritional compositions of foods would affect bacterial colonization in the colon, and that differences in bacterial colonization might affect RELMβ expression either directly or indirectly (*i.e.* systemic hormonal changes) through local Th2 cytokine production. Finally, a high-fat diet enlarges adipocytes, which in turn induces the secretion of various proteins such as TNFα while high-carbohydrate and high-protein diets reduce adipocyte size. In addition, FFA also reportedly induces the release of TNFα from macrophages [27]. TNFα secreted by adipocytes and macrophages would then induce the expression of RELMβ. We speculate that some or all of these mechanisms are involved in the nutrient-induced regulation of RELMβ. The high concentrations of RELMβ secreted by the intestines would reach the liver via the blood stream and thus contribute to the development of insulin resistance.

In conclusion, this study has clearly shown intestinal RELMβ expression to be strongly influenced by the nutritional compositions of foods. Up-regulation by inflammatory mediators, together with the previous demonstration of the RELMβ association with insulin resistance, suggests a role for this protein as a cytokine contributing to the pathogenesis of insulin resistance and thereby to that of the metabolic syndrome.

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