Oral administration of insulin-like growth factor-I from colostral whey reduces blood glucose in streptozotocin-induced diabetic mice

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Abstract

The aim of the present study was to investigate the effects of oral administration of the insulin-like growth factor-I-rich fraction (IGF-I-RF) from bovine colostral whey on the regulation of blood glucose levels in streptozotocin (STZ)-induced diabetic mice. We obtained a peptide fraction containing IGF-I (10 ng/mg protein) from Holstein colostrum within 24 h after parturition by using ultrafiltration. The blood glucose levels of STZ-induced diabetic mice fed with IGF-I-RF (50 μg/kg per d) were significantly reduced by 11 and 33 % at weeks 2 and 4, respectively ($P < 0.05$). The body weights of STZ-induced diabetic mice increased following the oral administration of the IGF-I-RF. The kidney weights of STZ-induced diabetic mice decreased significantly ($P < 0.05$) following the administration of the IGF-I-RF, and the liver weights of STZ-induced diabetic mice decreased significantly ($P < 0.05$) following the administration of 50 μg/kg per d of the IGF-I-RF. The present results indicate that the IGF-I-RF obtained from Holstein colostrum could be a useful component for an alternative therapeutic modality for the treatment of diabetes in insulin-resistant patients.

Key words: Insulin-like growth factor-I: Colostral whey: Blood glucose: Diabetes

Diabetes mellitus is a common and serious disorder caused by a reduction in insulin secretion from the pancreatic β-cells or by insulin resistance in organs. The incidence of diabetes has been steadily increasing worldwide. According to the WHO, in 2000, an estimated 150 million people above the age of 20 years suffered from diabetes, and this number is expected to increase to 300 million by 2025[1,2]. Insulin deficiencies lead to increased glucose concentrations in the blood, which can damage many of the body's systems resulting in vascular complications related to coronary artery disease and cerebrovascular disease[3–5]. The therapeutic measures for the treatment of hyperglycaemia include the use of insulin and other agents such as sulphonylureas and biguanides. These drugs may produce adverse side effects such as liver problems, lactic acidosis and diarrhoea[6,7]. For this reason, interest in evaluating natural products as potential treatments for diabetes has now increased in the scientific community[8,9].

Bovine colostrum, the initial milk produced by cows in the first 48 h after parturition, contains growth factors such as insulin-like growth factors (IGF)-I and II, transforming growth factor-β and epidermal growth factor, and pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α and interferon-γ[10–15]. IGF-I is a predominant growth factor present in concentrations of approximately 500 mg/l in bovine colostrum and of 18 μg/l in human breast milk[16,17]. IGF-I shares sufficient structural and functional homologies with insulin, suggesting that it may exert similar biological effects. IGF-I appears to exert some insulin-like effects via high-affinity binding with its own receptor. Thus, IGF-I has been widely investigated in connection with the development of potential therapeutic modalities for the treatment of diabetes, owing to the similarity of its metabolic actions to those of insulin[18,19]. Recombinant human IGF-I (RH IGF-I) has been suggested as a component of a potential treatment for diabetic patients suffering from the insulin-resistance syndrome[20,21]; however, the safety of RH IGF-I remains a concern. Therefore, we attempted to isolate IGF-I from bovine colostrum as a natural source instead of using RH IGF-I. Additionally, since, to the best of our knowledge, the hypoglycaemic activity of the IGF-I-rich fraction (IGF-I-RF) from bovine colostral whey has not been previously reported in experimental diabetes, the aim of the present study was to investigate potential hypoglycaemic effects

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using the IGF-I-RF from bovine colostral whey in streptozotocin (STZ)-induced diabetic mice.

Materials and methods

Insulin-like growth factor-I-rich fraction, recombinant human insulin-like growth factor-I and colostral whey

Similar to our previous study[22], in the present study, Holstein colostrum was used for the isolation of the IGF-I-RF and collected within 24 h after parturition. The colostrum samples were centrifuged (at 5000 rpm for 30 min at 4°C) to remove milk fat. The separated skimmed milk was subsequently acidified to pH 4·5 with 1 M-HCl and centrifuged (at 5000 rpm for 30 min at 4°C). According to the procedure described by Hossner & Yemm[23], the separated whey was gradually passed through 30 and 1 kDa ultrafiltration cartridges (Prep/Scale-TFF; Millipore, Billerica, MA, USA) to separate the IGF-I-RF, the free form of IGF-I (Fig. 1). IGF-I in the ultrafiltered fractions was verified using SDS-PAGE[24] and Western blotting[25]. IGF-I content was measured using sandwich ELISA[26]. RH IGF-I was purchased from R&D Systems (Minneapolis, MN, USA).

Experimental animals and administration of the insulin-like growth factor-I-rich fraction

Male imprinting control region mice (4 weeks old) were acquired from Orient (Seongnam, Korea), and five mice were randomly assigned to environmentally controlled, filtered cages (temperature 22°C; relative humidity 55 %) and maintained under 12 h light–12 h dark photocycle conditions. Mice were fed a standard diet (Purina Korea, Seongnam, Korea) during the 1-week adaptation period. Diabetes was induced in mice via an intraperitoneal administration of 100 μl STZ (Sigma Chemical Company, St Louis, MO, USA), which was dissolved in 0·01 M-citrate buffer (pH 4·5), at a dose of 45 mg/kg body weight (w/w). To prepare the control group, 100 μl of 0·01 M-citrate buffer (pH 4·5) were injected *in lieu* of STZ. Blood glucose levels were then measured using blood glucose measuring kits (YD Diagnostics, Yongin, Korea) 24 h after STZ administration. Blood samples were obtained from mice after they were deprived of food for 16 h. STZ-treated animals were considered diabetic when blood glucose levels exceeded 6·67 mmol/l. After induction of diabetes, mice were randomly divided into five groups (*n* 5 per group). Both non-diabetic and diabetes-induced groups received PBS daily for 4 weeks. IGF-I-RF, RH IGF-I and colostral whey containing 50, 5 and 0·5 μg protein/kg per d, respectively, were given daily for 4 weeks to diabetes-induced mice via oral administration. IGF-I-RF, RH IGF-I and colostral whey were dissolved in PBS, and administered to diabetes-induced mice in 200 μl doses. Blood samples were obtained once per week in the morning for 4 weeks (on 7, 14, 21 and 28 d). The animal experiments were conducted in accordance with the guidelines established by the Animal Care and Use Committee of the Rural Development Administration, and were approved by the committee.

Measurement of body weights

Body weights were measured daily and represented at 4 d intervals, beginning at the initiation of oral administration and continuing throughout the entire experimental period.

Measurement of organ weights

After 4 weeks, all mice were euthanised under deep anaesthesia with diethyl ether, and the heart, liver, spleen and kidney were weighed.
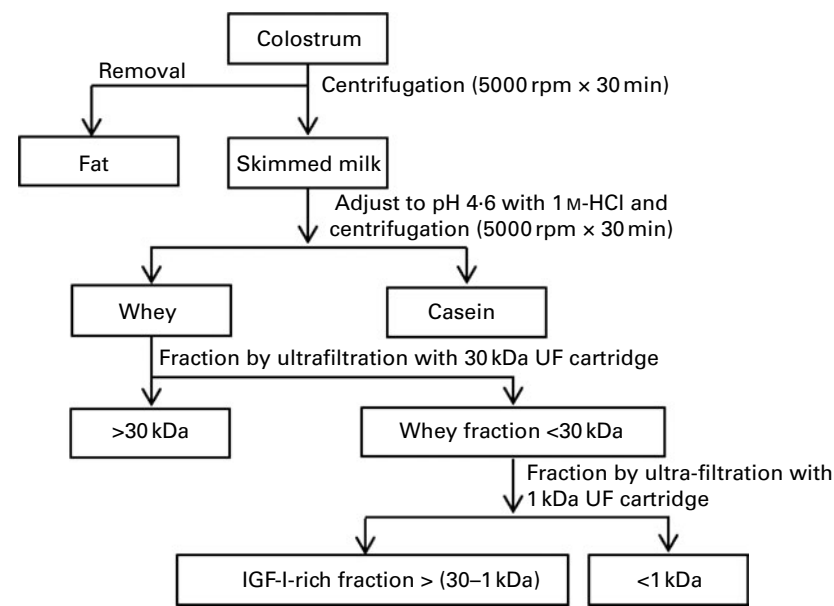
Fig. 1. Fractionation of the insulin-like growth factor (IGF)-I-rich fraction from Holstein colostrum using ultrafiltration (UF).

Evaluation of the insulin-like growth factor-I-rich fraction by the oral glucose tolerance test

Mice were divided into five groups (*n* 5) and received the following treatment: group I, PBS; group II, STZ + PBS; group III, STZ + IGF-I-RF (50 µg protein/kg); group IV, STZ + RH IGF-I (50 µg protein/kg); group V, STZ + colostral whey (50 µg protein/kg). Glucose (2·0 g/kg) was administered orally via needles. Blood was drawn from a tail vein at 0, 15, 30, 45, 60, 90 and 120 min.

Determination of blood glucose, insulin and tissue TAG levels

Blood glucose levels were measured using blood glucose measuring kits (YD Diagnostics). Blood insulin levels were measured by the insulin ELISA kit (Millipore). Tissue TAG was extracted using the Bligh & Dyer method[27] and measured using a Triglyceride Reagent (Diagnostic Chemicals Limited, Stamford, CT, USA).

Statistical analysis

Statistical analysis was performed with SPSS statistical software version 12.0. Descriptive statistics are expressed as means and standard deviations. One-way ANOVA was performed and when the values were significant ($P<0·05$), the differences of the mean values were determined using Duncan's multiple range tests.

Results

Identification of the insulin-like growth factor-I-rich fraction and insulin-like growth factor-I

The molecular weight of the recovered free IGF-I (7·6 kDa) was identified in the fraction obtained between 1 and 30 kDa ultrafiltration membranes by using SDS-PAGE and Western blotting (Fig. 2). IGF-I concentration in the IGF-I-RF obtained was 10 ng/mg protein.

Effects on blood glucose levels

Diabetes-induced mice were orally administered IGF-I-RF, RH IGF-I and colostral whey for 4 weeks. Blood glucose levels in the non-diabetic and STZ-induced diabetic groups fed with IGF-I-RF, RH IGF-I and colostral whey from 1 to 4 weeks are shown in Fig. 3. The IGF-I-RF and RH IGF-I groups had significantly reduced ($P<0·05$) blood glucose levels compared with the levels of the colostral whey group. The blood glucose levels returned to normal in a dose-dependent manner in the IGF-I-RF group, and these results were similar to the decreased blood glucose levels of the RH IGF-I group, which was used as the positive control.

Effects on body weights

The body weights of the STZ-induced diabetic control group decreased steadily during the entire experimental period (Fig. 4). However, the body weights of the IGF-I-RF, RH IGF-I and colostral whey groups increased slightly. Overall, the body-weight gain increased more effectively in the RH IGF-I or IGF-I-RF group than in the group fed with bovine colostral whey.

Effects on organ weights

The organ weights of the individual groups are shown in Table 1. Heart weights of all experimental groups were similar to those of the normal group. Kidney, spleen and liver weights of the STZ-induced diabetic group were significantly ($P<0·05$) increased compared with those of the normal group. Kidney and spleen weights for the IGF-I-RF, RH IGF-I and colostral whey groups were significantly ($P<0·05$) reduced. The liver weights of the IGF-I-RF and RH-IGF-I groups were reduced and were similar to those of the control group, and this dose-dependent reduction was significant ($P<0·05$). No significant differences in the colostral whey group were observed.
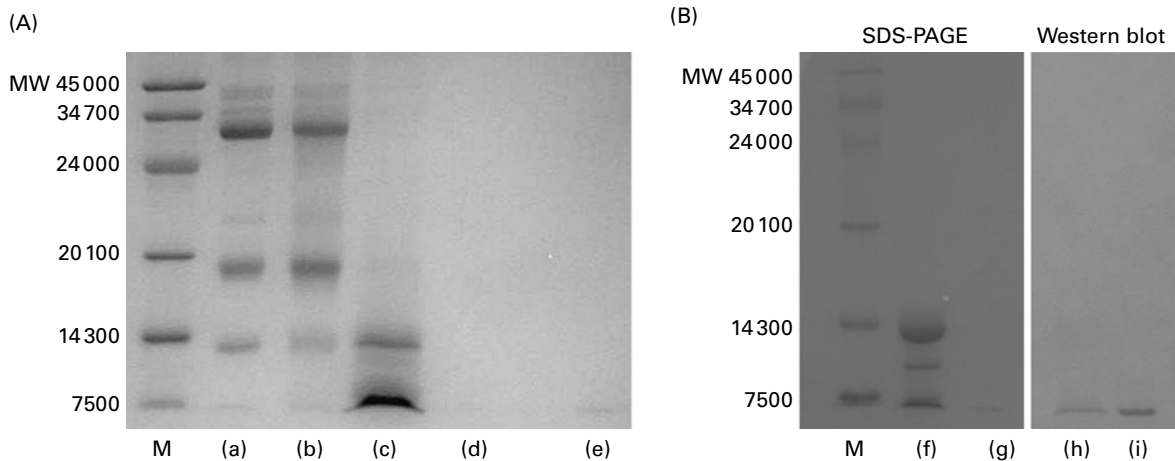
Fig. 2. (A) SDS-PAGE patterns of isolated fractions from colostral whey and (B) SDS-PAGE and Western blot patterns of the insulin-like growth factor (IGF)-I-rich fraction. MW, molecular weight; M, marker. (a) Whey, (b) >30 kDa fraction, (c) 1−30 kDa fraction, (d) <1 kDa fraction, (e) standard IGF-I, (f) 1−30 kDa fraction, (g) standard IGF-I, (h) 1−30 kDa fraction and (i) standard IGF-I.
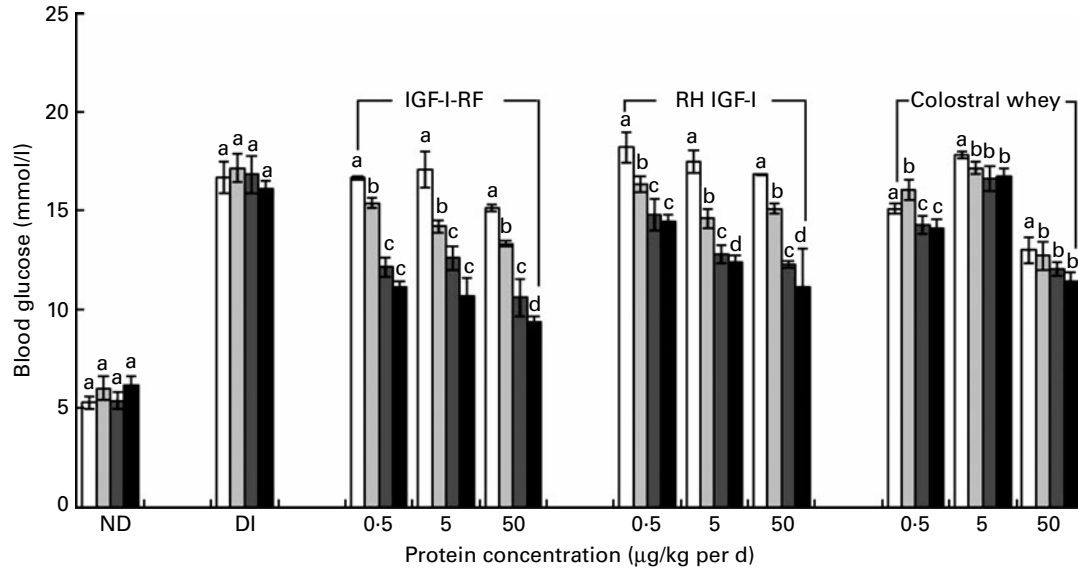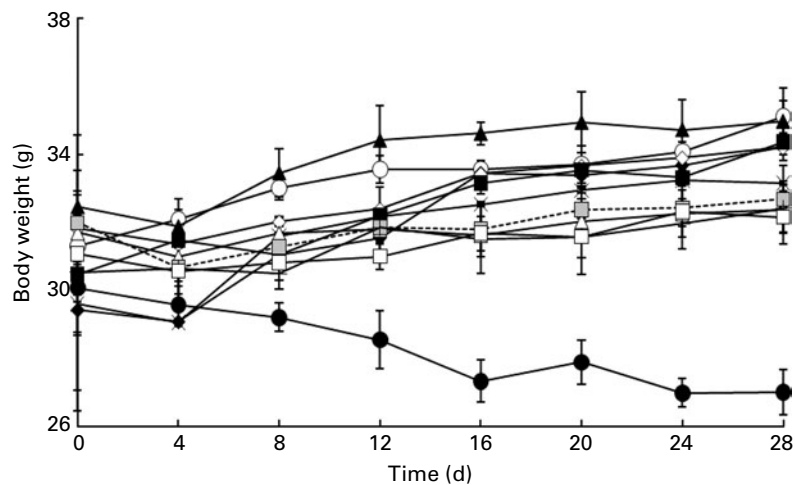
Fig. 3. Effects of insulin-like growth factor-I-rich fraction (IGF-I-RF), recombinant human IGF-I (RH IGF-I) and colostral whey on blood glucose levels in streptozotocin-induced diabetic mice. ND, non-diabetic mice; DI, diabetes-induced mice. [a,b,c,d] Mean values with unlike letters for the same sample and level of protein concentration were significantly different ($P<0.05$). ▢, 1 week; ▨, 2 weeks; ▤, 3 weeks; ▪, 4 weeks.

Effects of the insulin-like growth factor-I-rich fraction on the oral glucose tolerance test

In the oral glucose tolerance test, the highest rise in blood glucose occurred in STZ-induced control mice at 30 min after oral glucose ingestion and decreased slowly after 30 min (see Supplementary data 1 of the supplementary material, available online at http://www.journals.cambridge.org/bjn). The IGF-I-RF and RH IGF-I significantly suppressed the increase in blood glucose in comparison with STZ ($P<0.05$). The IGF-I-RF and RH IGF-I led to a decrease in blood glucose levels by 16·70 (SD 1·49) and 15·81 (SD 3·07) mmol/l at 60 min, and by 13·93 (SD 1·06) and 13·17 (SD 1·58) mmol/l at 120 min, respectively.

Effects of the insulin-like growth factor-I-rich fraction on blood insulin and tissue TAG levels

The effects of the IGF-I-RF on blood insulin and tissue TAG levels in the STZ-induced diabetic group are shown in Supplementary data 2 of the supplementary material (available online at http://www.journals.cambridge.org/bjn). Blood insulin levels significantly increased in the IGF-I-RF, RH IGF-I and colostral whey groups compared with those in the STZ-induced diabetic group ($P<0.05$). Intracellular concentrations of TAG decreased in the liver of IGF-I-RF- and RH IGF-I-treated mice compared with those in STZ-induced diabetic mice; however, the difference was not significant.

Fig. 4. Effect on the body weight of streptozotocin-induced diabetic mice fed with insulin-like growth factor-I-rich fraction (IGF-I-RF), recombinant human IGF-I (RH IGF-I) and colostral whey. ─○─, Non-diabetic mice; ─●─, diabetes-induced mice; ─△─, IGF-I-RF (0·5); ─▲─, RH IGF-I (0·5); ─□─, colostral whey (0·5); ─■─, IGF-I-RF (5); ─✕─, RH IGF-I (5); ─✛─, colostral whey (5); ─◇─, IGF-I-RF (50); ─◆─, RH IGF-I (50); ··▫··, colostral whey (50).

Table 1. Effects of insulin-like growth factor-I-rich fraction (IGF-I-RF), recombinant human IGF-I (RH IGF-I) and colostral whey on the organ weights of streptozotocin-induced diabetic mice
(Mean values and standard deviations)

Group	Dose (μg/kg per d)	Body weight (g)	Relative organ weight (g/100 g body weight)*							
			Heart		Kidney		Liver		Spleen	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Non-diabetic mice	–	35·11[a]	0·42	0·05	1·529[a]	0·1	3·982[a]	1·04	0·347[a]	0·06
Diabetes-induced mice	–	27·01[c]	0·56	0·15	2·284[b]	0·32	6·061[c]	0·71	0·703[b]	0·19
IGF-I-RF†	0·5	34·97[a]	0·43	0·05	1·707[a]	0·15	4·578[a,b]	0·89	0·352[a]	0·45
	5	33·16[b]	0·46	0·2	1·707[a]	0·73	4·789[a,b]	0·26	0·365[a]	0·09
	50	34·22[a]	0·43	0·05	1·616[a]	0·46	4·509[a,b]	0·46	0·348[a]	0·11
RH IGF-I†	0·5	32·36[b]	0·47	0·06	1·740[a]	0·09	4·713[a,b]	1·02	0·386[a]	0·07
	5	34·36[a]	0·43	0·07	1·548[a]	0·45	4·395[a,b]	0·66	0·343[a]	0·07
	50	34·21[a]	0·44	0·08	1·520[a]	0·11	4·209[a]	1·47	0·362[a]	0·11
Colostral whey†	0·5	32·16[b]	0·45	0·03	1·903[a]	0·16	5·311[c]	1·02	0·339[a]	0·04
	5	32·41[b]	0·46	0·04	1·898[a]	0·42	5·239[b,c]	1·44	0·364[a]	0·06
	50	32·68[b]	0·46	0·07	1·845[a]	0·28	5·233[b,c]	0·69	0·367[a]	0·08

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).
* The organ weights were expressed as the ratio of body weight:organ weight.
† After diabetes was induced, mice were orally administered IGF-I-RF, RH IGF-I and colostral whey for 4 weeks.

Discussion

Current research worldwide is aimed at developing bioactive food and new drug materials from natural resources. Along similar lines, our research is directed towards exploring food materials that have the potential to regulate metabolic syndromes such as diabetes by using natural ingredients or waste materials. In the present study, we hypothesised that the IGF-I-RF from colostral whey could imitate insulin function. To evaluate this, the IGF-I-RF was isolated and purified from the colostrum, and its antidiabetic activity was analysed by measuring blood glucose levels, body weight, organ weight and blood insulin levels.

The results of several studies[21,28–30] suggested that RH IGF-I is involved in the regulation of metabolic control in patients with type 2 diabetes mellitus via a reduction in insulin resistance by decreasing blood glucose, serum TAG and total cholesterol levels. Moreover, 24 h creatinine clearance increases when RH IGF-I is injected into diabetic patients. Therefore, we isolated IGF-I from the colostrum as a natural source, instead of using RH IGF-I, and confirmed its effects on blood glucose reduction through oral administration of the isolated IGF-I-RF in mice.

A number of previous studies have focused on the effects of RH IGF-I in type 2 diabetic patients[21,31–33]. RH IGF-I (120 μg/kg) was subcutaneously injected into type 2 diabetic patients twice per d for 5 d, and this resulted in a 2·5-fold elevation of free IGF-I above basal levels. The fasting blood glucose level decreased by 30 %, whereas insulin and C-peptide levels decreased by 50 %, suggesting that RH IGF-I attenuated insulin resistance[31]. Schalch et al.[21] used doses of RH IGF-I as high as 160 μg/kg in twelve patients and reported improvements in fasting glucose, insulin and C-peptide levels. The mechanism by which IGF-I improves glycaemic control and insulin sensitivity remains to be elucidated. The observed reductions in fasting blood glucose levels suggest that RH IGF-I exerts a significant effect on the production of

hepatic glucose, which is the principal determinant of fasting glucose levels[34]. In addition, IGF-I also enhances glucose output, increasing fatty acid oxidation and glucose uptake in skeletal muscles[35,36]. The present results also showed that the orally administered IGF-I-RF improved blood glucose levels in STZ-induced diabetic mice; a trend very similar to that observed with pure IGF-I (RH IGF-I) was evident. Orally administered bovine colostral whey also reduced blood glucose levels in STZ-induced diabetic mice. Therefore, bovine colostral IGF-I may be used to enhance insulin sensitivity in healthy subjects and may prove particularly effective in the treatment of insulin-resistant diabetics.

The lack of insulin synthesis due to the damage of pancreatic β-cells by STZ injection induces weight loss via an imbalance in glucose metabolism[37,38]. In the present study, we showed that all STZ-induced diabetic mice lost weight in the first 4 d following STZ, but all treatments (IGF-I-RF, RH IGF-I and colostral whey) resulted in weight gain beginning 5 d after the initial oral administration. We summarise that the weight loss observed in STZ-induced diabetic mice beginning 4 d after oral ingestion of the aforementioned substances may have been attributable to the lack of insulin secretion and fat degradation induced by the destruction of pancreatic β-cells, as has been concluded in the previous studies of Smith et al.[39] and Kadowaki et al.[40]. With regard to the present results, the recovery of body weights in STZ-induced diabetic mice may have also been attributable to absorption of the orally administered IGF-I-RF from bovine colostral whey. Therefore, oral supplementation with colostral whey and the IGF-I-RF may prove useful in preventing body-weight reduction in subjects suffering from diabetes mellitus.

Diabetes-associated liver hypertrophy was attributable to a lack of insulin synthesis, resulting in a failure to metabolise ingested glucose and subsequent storage of lipids within the liver[37]. In the present study, an increase in liver weights was also observed in STZ-induced diabetic control mice. However, the liver weights of non-diabetic mice were statistically

identical to those of mice fed RH IGF-I and were lower in mice fed the IGF-I-RF than in STZ-induced diabetic control mice. The liver weights of STZ-induced diabetic mice fed with 50 μg/kg per d of the IGF-I-RF did not significantly differ from those of normal mice. Thus, the present results indicate that the administration of the IGF-I-RF obtained from colostral whey exerted an ameliorative effect on liver hypertrophy associated with diabetes mellitus.

In addition, the kidney weights of the STZ-induced diabetic group were significantly ($P<0·05$) higher than those of the normal group, and kidney weights significantly ($P<0·05$) decreased in the IGF-I-RF, RH IGF-I and colostral whey groups.

Sochor et al.[41] reported that orally administered RH IGF-I significantly reduced the kidney weights in STZ-induced diabetic mice and observed a positive relationship between kidney hypertrophy and hyperglycaemia in diabetic mice. STZ-induced diabetic rats also have been shown to have larger kidneys than non-diabetic rats[42]. The rate of kidney hypertrophy over the first 7 d of induced diabetes in mice was correlated with blood glucose concentrations. Over a wide range of blood glucose concentrations (6·44–18·89 mmol/l), kidney weights, protein contents and protein:DNA ratios were closely correlated with blood glucose levels[42]. Alternatively, it has been reported that a high concentration of glucose metabolised into UDP-galactose or glycogen is a cause of cell membrane hypertrophy. Subsequently, glycogen stored in mesangial cells of the glomerulus results in kidney hypertrophy[43].

The findings of the present study are particularly interesting because oral administration of a peptide-enriched fraction with a molecular weight corresponding to that of IGF-I significantly reduced blood glucose levels in a STZ-induced diabetic mouse model. These peptide fractions also improved the weights of kidney and liver in STZ-induced diabetic mice. The trend towards a decrease in blood glucose levels in STZ-induced diabetic mice after feeding was similar to that observed with the administration of insulin. Therefore, the present study confirmed that the oral consumption of fractions including IGF helps in controlling blood glucose levels in patients with diabetes mellitus. However, future studies and clinical trials are required to investigate the metabolic mechanism related to blood glucose reduction.

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