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University of Leeds
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E-Mail: L.Dye@leeds.ac.uk

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Department of Epidemiology & Biostatistics
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Marie Lewis
Department of Food & Nutritional Sciences
University of Reading
RG6 6AP
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E-Mail: marie.lewis@reading.ac.uk

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Hollie Raynor
Public Health Nutrition
Institute of Biomedical Engineering
University of Tennessee
Knoxville, USA
E-Mail: hraynor@utk.edu

Obesity
Hollie Raynor
Public Health Nutrition
Institute of Biomedical Engineering
University of Tennessee
Knoxville, USA
E-Mail: hraynor@utk.edu

Phytochemicals
Chris Gill
Northern Ireland Centre for Food and
Health; Institute of Biomedical Sciences
University of Ulster
Coleraine, UK
E-Mail: C.gill@ulster.ac.uk

Regulation of food intake, satiety and gut hormones
Inger Björck
Food for Health Science Centre
Lund University
Lund, Sweden
E-Mail: Inger.Bjorck@food-health-science.lu.se

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ORIGINAL CONTRIBUTION

Deteriorated glucose metabolism with a high-protein, low-carbohydrate diet in *db* mice, an animal model of type 2 diabetes, might be caused by insufficient insulin secretion

Emi Arimura[1,2] · Wijang Pralampita Pulong[2] · Ancah Caesarina Novi Marchianti[2,4] · Miwa Nakakuma[2,3] · Masaharu Abe[2] · Miharu Ushikai[2] · Masahisa Horiuchi[2]

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Abstract
Purpose We previously showed the deleterious effects of increased dietary protein on renal manifestations and glucose metabolism in leptin receptor-deficient (*db*) mice. Here, we further examined its effects on glucose metabolism, including urinary C-peptide. We also orally administered mixtures corresponding to low- or high-protein diets to diabetic mice.
Methods In diet experiments, under pair-feeding (equivalent energy and fat) conditions using a metabolic cage, mice were fed diets with different protein content (L diet: 12 % protein, 71 % carbohydrate, 17 % fat; H diet: 24 % protein, 59 % carbohydrate, 17 % fat) for 15 days. In oral administration experiments, the respective mixtures (L mixture: 12 % proline, 71 % maltose or starch, 17 % linoleic acid; H mixture: 24 % proline, 59 % maltose or starch, 17 % linoleic acid) were supplied to mice. Biochemical parameters related to glucose metabolism were measured.
Results The *db–H* diet mice showed significantly higher water intake, urinary volume, and glucose levels than *db–L* diet mice but similar levels of excreted urinary C-peptide. In contrast, control-H diet mice showed significantly higher C-peptide excretion than control-L diet mice. Both types of mice fed H diet excreted high levels of urinary albumin. When maltose mixtures were administered, *db–L* mixture mice showed significantly higher blood glucose after 30 min than *db–H* mixture mice. However, *db* mice administered starch–H mixture showed significantly higher blood glucose 120–300 min post-administration than *db–L* mixture mice, although both groups exhibited similar insulin levels.
Conclusions High-protein, low-carbohydrate diets deteriorated diabetic conditions and were associated with insufficient insulin secretion in *db* mice. Our findings may have implications for dietary management of diabetic symptoms in human patients.

Keywords High-protein diet · Insulin secretion · *db* mice · Oral administration · Urinary C-peptide

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✉ Masahisa Horiuchi
masakun@m.kufm.kagoshima-u.ac.jp

[1] Major in Food and Nutrition, Department of Life and Environmental Science, Kagoshima Prefectural College, 1-52-1 Shimo-Ishiki, Kagoshima 890-0005, Japan

[2] Department of Hygiene and Health Promotion Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

[3] Takata Hospital, 5-1 Horie-cho, Kagoshima 892-0824, Japan

[4] Present Address: Medical Faculty of Jember University, Kalimantan Street 37, East Java, Indonesia

Introduction

Recently increasing numbers of diagnosed diabetes mellitus (DM) and diabetic nephropathy cases worldwide are more likely related to lifestyle than to genetic factors, as global genetic diversity has not changed appreciably over this short period of time [1–3]. Lifestyle factors, including nutrition, have markedly shifted in the past several decades, although the impact of altered nutrition on development of DM and diabetic nephropathy is not fully understood and remains controversial [4–6]. Initial diets of many Japanese individuals, such as second-generation Japanese-American

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men, have since conformed to modern Western diets, resulting in increased consumption of lipids and animal protein but decreased intake of carbohydrates and dietary fibre. This nutritional change might be implicated in the onset and development of DM [7]. While dietary fat is a recognized factor in deteriorating insulin sensitivity, dietary protein may stimulate insulin secretion, leading to exhaustion of β-cell function in the pancreas, although this relationship is also controversial [6, 8].

To limit DM in Japanese individuals with decreased insulin secretion capacity [9], we should consider how their diet affects development of DM and diabetic nephropathy. Therefore, we previously examined the effect of clinically relevant dietary protein content diets (12–24 % energy) on glucose levels and renal manifestations in *db* mice, an animal model for DM exhibiting leptin receptor deficiency and a relatively low capacity for insulin secretion [10, 11]. We found that a high-protein, low-carbohydrate diet increased blood glucose levels and deteriorated renal manifestations, in contrast to a low-protein, high-carbohydrate diet [12]. Similarly, a human study revealed that a high-protein diet led to highly advanced glycation, often resulting in HbA1c production [13]. In some diabetic cases, deterioration of blood glucose levels appears linked to low-carbohydrate, high-protein diets [14], although the pathophysiological mechanism involved is still unclear.

Therefore, in the present study, we evaluated the effects of dietary protein content on glucose metabolism in young *db* mice without severe diabetic complications under pair-feeding and metabolic cage conditions. Additionally, we examined levels of blood glucose and insulin after the oral administration to *db* and control mice of mixtures corresponding to low-protein or high-protein diets and containing different types of carbohydrates. Our findings provide evidence of deteriorating glucose metabolism in diabetic animals due to the consumption of a high-protein diet that may be linked to insufficient insulin secretion.

Materials and methods

Animals

Four-week-old male diabetic *db* mice [C57BLKS(BKS). Cg-+ *Lepr^{db}*/+ *Lepr^{db}*/J] with a homozygous mutation in the leptin receptor gene and non-diabetic control (CT) mice (BKS.Cg-Dock7^{m} +/ Dock7^{m} +/J) were purchased (Charles River Japan, Kanagawa, Japan) [15]. The mice were housed individually with a humidity- and temperature-controlled (50 ± 10 %, 22 ± 2 °C) facility under a 12-h light/dark cycle (0700–1900 h). The mice had ad libitum access to water.

Food and water intake of mice in metabolic cages

For pair-feeding metabolic cage experiments, 8 CT and 16 *db* mice were randomly separated and housed individually in cages (3600M021, Tecniplast Japan, Co., Ltd., Tokyo, Japan) for 3 days and received one of the two protein diets: 12 % (low protein; L) or 24 % (high protein; H) protein composed of 50 % animal and 50 % plant protein (Supplementary Table 1). For the pair-fed experiments, we first measured the amount consumed by the control mice that were fed the L diet ad libitum. Control mice that were fed the H diet ad libitum consumed a similar amount of food. The total amount of food consumed was 48.5 g (mean) in 15 days; therefore, we supplied 3.23 g per day to the *db* mice. After 3 days of acclimation, *db–H* and *db–L* mice were supplied diets for 15 days equivalent to amounts consumed by CT-L mice housed individually in metabolic cages [16] with ad libitum access to food. The body weight (BW) of each mouse was measured between 0700 and 0900 h on the 7th and 14th days. Water intake was measured once per week. Urine was collected and measured from 0700 to 0700 h at days 6–7 and 13–14 and stored at −80 °C for later analysis. On the final day (day 15), mice were anesthetized by pentobarbital (100 mg/kg) after 6-h fasting (0700–1300 h), and blood was collected from the hearts. The blood was mixed with EDTA (final concentration of 4 mM) and centrifuged, and the supernatant was stored at −80 °C for later analysis. Organs, including the kidneys, heart, liver, and fat surrounding the epididymis were weighed.

Measurement of blood glucose and insulin levels after oral administration of dietary mixtures with different protein content

Sixteen CT and 16 *db* mice at 4 weeks of age were housed individually for 1 week and received a standard diet (CE2: 29 % protein; CLEA Japan, Shizuoka, Japan). Subsequently, the mice were randomly separated and fasted overnight (1400–0800 h). The next day, mice were orally administered one of the dietary mixtures corresponding to an L diet or H diet with maltose as the carbohydrate source (Table 1) at 5.0 ml/kg body weight. Blood glucose was measured from the tail veins of mice with a glucose metre (FreeStyle Freedom; Nipro Corp., Osaka, Japan) before and at 15, 30, 60, 120, 240, and 300 min after the oral administration of the respective dietary mixture. After 1-week acclimation, the mice were randomly separated and fasted overnight (1400–0800 h). The next day, mice were orally administered one of the dietary mixtures with soluble starch as the carbohydrate source (Table 1), as well as maltose administration experiments. Insulin was measured

Table 1 Composition of the three major nutrients in the mixtures used in the oral administration test

	L	H
A		
	Maltose mixture	
Proline[a] (g)	1.33 (12)	2.66 (24)
Maltose[b] (g)	7.83 (71)	6.50 (59)
Linoleic acid[c] (g)	0.84 (17)	0.84 (17)
Total (g)	10.0	10.0
H_2O (ml)	10.0	10.0
Energy (kcal/ml)	2.21	2.21
B		
	Starch mixture	
Proline[a] (g)	1.33 (12)	2.66 (24)
Starch[d] (g)	7.83 (71)	6.50 (59)
Linoleic acid[c] (g)	0.84 (17)	0.84 (17)
Total (g)	10.0	10.0
H_2O (ml)	10.0	10.0
Energy (kcal/ml)	2.21	2.21

The number within parentheses indicates per cent of energy provided

The mixture is comprised of 50 % water

[a] Proline (Wako Pure Chemical Industries)

[b] Maltose (Wako Pure Chemical Industries)

[c] Linoleic acid (Wako Pure Chemical Industries)

[d] Starch (Wako Pure Chemical Industries)

in other 10 CT and 9 *db* mice after 1-week acclimation using plasma prepared from EDTA-treated blood collected from the mice's tail veins before and at 15, 60, 150, and 240 min after oral administration of the dietary mixture.

Biochemical measurements

Blood glucose and urinary glucose were measured by a commercial kit (glucose CII-test Wako; WAKO, Tokyo, Japan) according to the manufacturer's instructions. Leptin, insulin, urinary C-peptide, and urinary albumin were measured by their respective ELISA kits (R&D Systems, Minneapolis, MN; Morinaga Institute of Biological Science Inc., Kanagawa, Japan; Yanaihara Institute Inc., Shizuoka, Japan; Exocell Inc., Philadelphia, PA). Creatinine clearance (Ccr) was calculated by the following equation: Ccr (ml/h) = [Urine volume (ml/day) × urinary creatinine (mg/ml)]/[24 (h) × blood creatinine (mg/ml)]. Creatinine was measured by a kit based on a reaction of creatininase (CRE-EN; Kainos Lab. Inc., Tokyo, Japan).

Statistical analysis

Values are shown as mean ± standard error (SE). Statistical analysis was performed using the one-way or two-way (repeated measurement) analysis of variance (ANOVA) as appropriate. Significant differences were determined using Fisher's PSD test for multiple comparisons or an unpaired Student's *t* test. $P < 0.05$ indicated statistical significance (Ekuseru-Tokei 2010; Social Survey Research Information, Tokyo, Japan).

Results

Body weight and renal manifestations of mice under pair-feeding and metabolic cage conditions

The CT-H mice consumed similar amounts of food to CT-L mice, although the CT-L mice had ad libitum access to food. While the *db* mice showed significantly higher BW than the CT mice fed their respective diets (Fig. 1a), for both types of mice there was no significant effect of diet type on BW. As shown in Fig. 1b–d, *db* mice also showed significantly higher amounts of water intake, urinary volume, and glucose than CT mice fed their respective diets. Although both CT groups showed similar measurements, *db–H* mice showed significantly higher levels of all three parameters than *db–L* mice. Furthermore, Fig. 1e shows that *db* mice had significantly higher urinary albumin levels than CT mice according to each diet and that all H diet mice, regardless of the genotype, showed significantly higher urinary albumin levels than the L diet mice. Creatinine clearance, another indicator of kidney function, was significantly lower in *db* mice than in CT mice under both diet conditions (Table 2), but we observed no significant difference in the Ccr of the *db* and CT mice with respect to each diet group. In addition, the *db–L* mice excreted significantly higher levels of urinary C-peptide than the CT-L mice, and although the CT-H mice exhibited increased excretion of urinary C-peptide compared to the CT-L mice, we saw no significant difference in urinary C-peptide excretion between the *db–L* and *db*–H mice (Fig. 1f).

Kidney weight was significantly lower in *db* mice than in CT mice and significantly higher in *db–H* mice than in *db–L* mice (Table 2). Although CT-H mice had higher-weight kidneys than CT-L mice, the difference did not reach significance ($P = 0.28$). We also weighed other organs and tissues, including the liver, white adipose tissue (WAT), and the heart. These measurements revealed that *db* mice had higher-weight livers and fat surrounding the epididymis but significantly lower-weight hearts than CT mice according to each diet. However, there was no significant effect of diet type on the weight of the liver, WAT, or heart for the two groups.

Hormone levels and biochemical parameters of blood and urine samples

We examined fasting blood glucose (FBG), leptin, and insulin levels in *db* and CT mice under metabolic cage

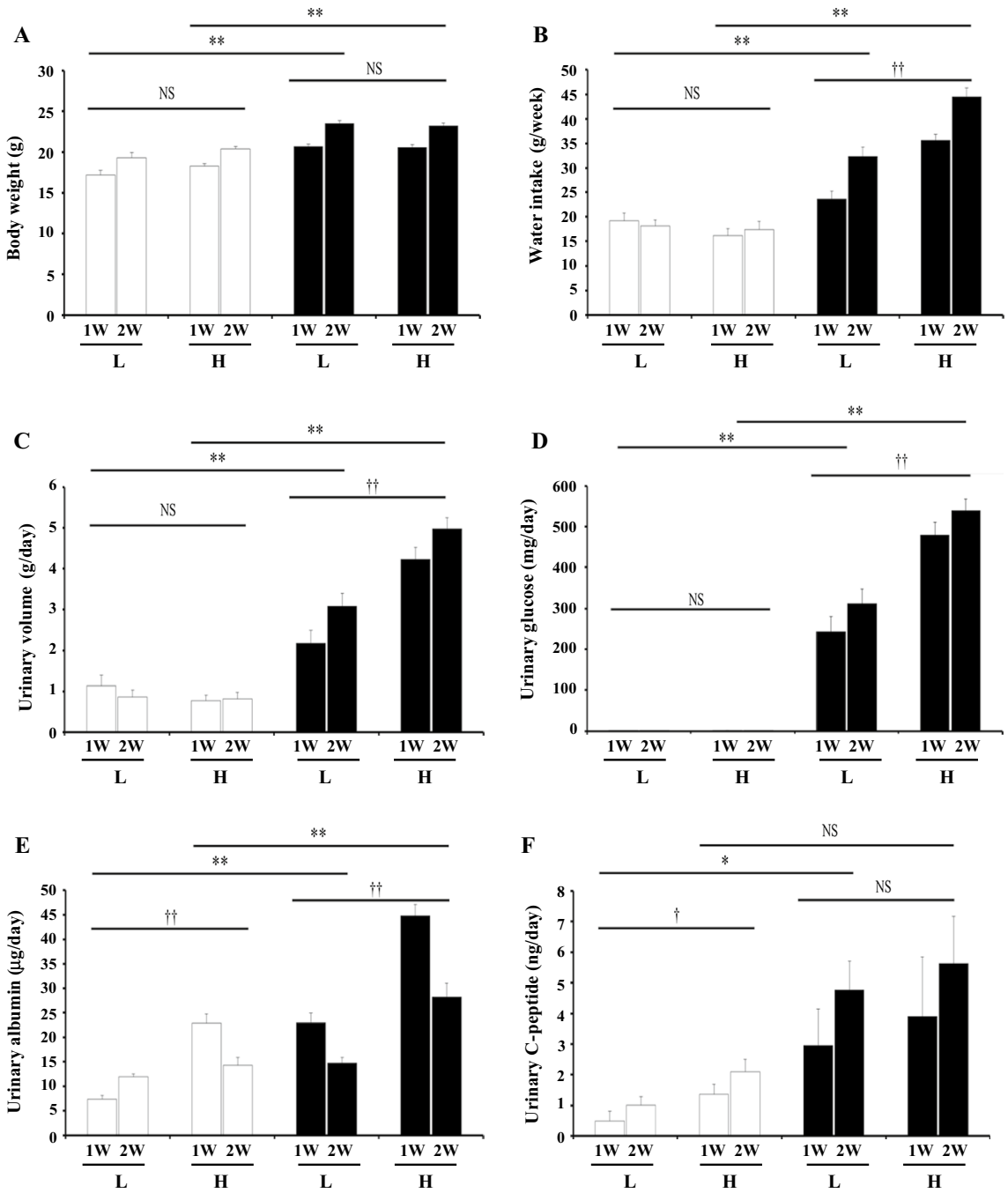

Fig. 1 Under pair-feeding and metabolic cage conditions, body weight (**a**), water intake (**b**), urinary volume (**c**), urinary glucose (**d**), urinary albumin (**e**), and urinary C-peptide (**f**) of CT (*open column*) and *db* (*closed column*) mice fed with different diets during 2 weeks are shown. Data are presented as means + SE from 4 CT and 8 *db* mice under the respective conditions and were analysed by two-way ANOVA (repeated measurements). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with CT mice fed with the respective diet. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with same-genotype mice fed the L diet. *NS* not significant, *CT* control, *db* leptin receptor deficient

condition. For FBG, *db–L* mice showed significantly higher values than *db–H* mice, but we saw no significant difference in FBG between CT mice fed with the respective diets. Similarly, *db* mice showed significantly higher insulin levels than CT mice with respect to each diet, while insulin levels within the *db* and CT groups did not significantly differ between diets. The *db–L* mice exhibited significantly higher leptin levels than

Table 2 Effects of diets on organ weights, blood, and urinary parameters in CT and *db* mice

Diet	CT		*db*	
	L	H	L	H
n	4	4	8	8
Organ weight (g)				
Kidney	0.28 ± 0.02	0.30 ± 0.01	0.21 ± 0.00**	0.26 ± 0.00**,##
Liver	0.76 ± 0.02	0.84 ± 0.03	1.00 ± 0.02**	1.01 ± 0.02**
WAT	0.29 ± 0.02	0.33 ± 0.05	0.97 ± 0.02**	0.93 ± 0.02**
Heart	0.100 ± 0.003	0.104 ± 0.004	0.080 ± 0.001**	0.082 ± 0.001**
Blood parameters				
FBG (mg/dl)	138 ± 18	112 ± 26	118 ± 16	74 ± 10#
Insulin (μg/l)	0.6 ± 0.2	0.5 ± 0.3	4.6 ± 0.9*	4.1 ± 0.7**
Leptin (μg/l)	2.6 ± 0.3	3.7 ± 1.6	29.3 ± 6.3*	47.4 ± 14.0
Urinary parameter				
Ccr (ml/h)	3.5 ± 0.2	2.8 ± 0.4	1.3 ± 0.1**	1.3 ± 0.1**

Values are mean ± SE. Values were analysed by Student's *t* test

FBG fasting blood glucose, *Ccr* creatinine clearance, *WAT* white adipose tissue

* $P < 0.05$; ** $P < 0.01$ compared with CT mice fed the respective diet

# $P < 0.05$; ## $P < 0.01$ compared with the respective genotype mice fed L diet

CT-L mice, but leptin levels did not differ significantly within the *db* or CT groups fed each diet. Hormone and FBG measurements are summarized in Table 2.

Effect of maltose and soluble starch on glucose and insulin levels in CT and *db* mice

To determine the cause of higher urinary glucose levels found in *db–H* mice compared to *db–L* mice, we examined changes in blood glucose after administration of the various diet mixtures (Table 1). The *db* mice administered the L–maltose mixture showed significantly higher blood glucose values than *db* mice administered the H–maltose mixture after 30 min post-administration (354 ± 49 vs. 206 ± 32, $P < 0.05$) (Fig. 2A1). We observed no significant differences in blood glucose between CT mice and *db* mice after administration of the H and L mixtures containing maltose at 120–300 min (Fig. 2A1, A2). In contrast, although *db–H* mice administered a soluble starch mixture showed similar blood glucose levels to *db–L*–starch mixture mice after 30 min post-administration (Fig. 2B1), the *db–H*–starch mixture mice showed significantly higher blood glucose levels than *db–L*–starch mixture mice during 120–300 min post-administration (Fig. 2B1, B2). The *db* mice administered the mixtures containing starch showed higher blood glucose from 120 to 300 min than the control mice administered the H or L mixtures. Although we did not measure urinary glucose in this experiment, the higher blood glucose might be reflected in the urinary glucose excretion.

As shown in Fig. 2C, *db* mice administered both soluble starch mixtures had higher blood insulin levels at 0–240 min post-administration than CT mice administered the corresponding mixtures. Notably, we saw no significant difference in blood insulin levels between mice administered the H–starch or L–starch mixtures within the *db* or CT groups.

Discussion

Our study revealed that high-protein diets increased urinary output and glucose levels under metabolic cage conditions. Urinary glucose excretion increased if the protein/carbohydrate ratio in the diet increased, while insulin excretion, measured as urinary C-peptide, was unaffected. This finding was only observed in the *db* mice. The control mice might produce additional insulin to compensate for the H diet. On the other hand, *db* mice cannot produce adequate insulin, as shown by the high glucose concentration in the blood. That difference might be due to the capacity of the islets to secrete insulin, implying that the toxicity of the H diet in terms of glucose metabolism might be related to residual insulin capacity.

The deterioration of glucose metabolism resulting from the H diet was further evidenced by increased blood glucose and constant insulin levels in *db* mice following the administration of a high-proline mixture with low amounts of starch-based carbohydrates. Thus, a high-protein, low-carbohydrate diet composed of polysaccharides, such as starch, worsens metabolic symptoms in diabetic mice.

To examine the relationship between diet and insulin secretion, we collected urine from young mice under pair-feeding and metabolic cage conditions without

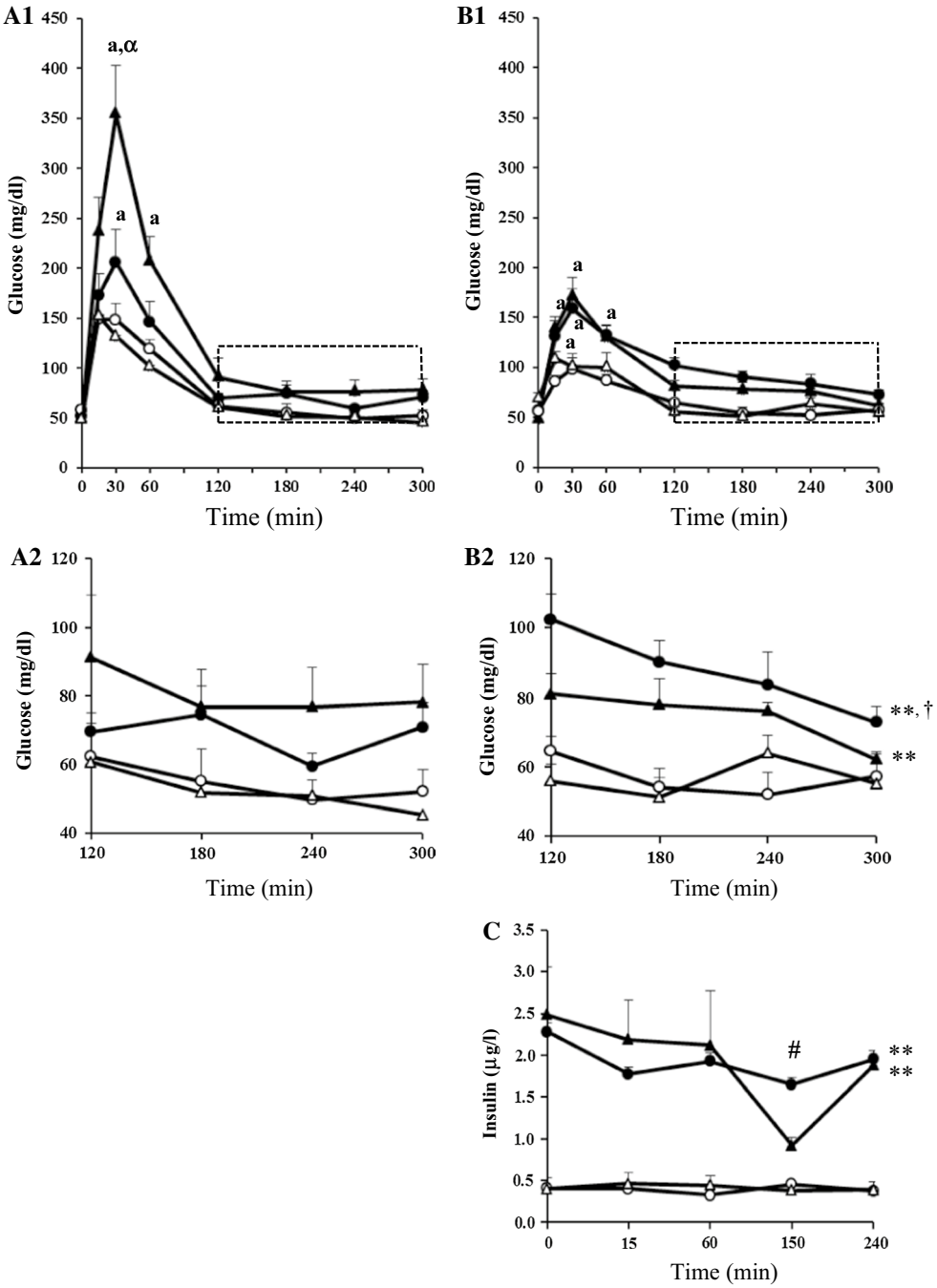

Fig. 2 Oral administration of mixtures composed of maltose (**A**) or soluble starch (**B**), corresponding to low-protein or high-protein diets. Levels of blood glucose in CT (*open column*) and *db* (*closed column*) mice are shown after administration of each dietary mixture (L mixture, *triangle*; H mixture, *circle*) during 0–300 min. Data are presented as means + SE from 7 to 8 CT and 8 *db* mice under their respective conditions. The *squares* enclosed by the *dotted lines* in **A1** and **B1** are enlarged as **A2** and **B2**. **C** Levels of blood insulin in CT (*open column*) and *db* (*closed column*) mice are shown after the administration of each dietary mixture (L mixture, *triangle*; H mixture, *circle*) during 0–240 min. The data are presented as means + SE from 5 CT and 4–5 *db* mice under their respective conditions. In **A1** and **B1**, data obtained at the same time after administration were analysed with an unpaired Student's *t* test. The Roman letter "*a*" indicates a significant difference, $P < 0.05$ versus CT mice with the corresponding mixture. The Greek letter "*α*" indicates a significant difference, $P < 0.05$ versus *db* mice with the administration of H mixture. In **A2**, **B2**, and **C**, the data were analysed by two-way ANOVA (repeated measurement). $^{**}P < 0.01$, for CT versus *db* mice with the respective mixtures containing starch. $^{\dagger}P < 0.05$, for *db* mice with the administration of L and H mixtures

severe secondary complications, such as decreased BW. In our previous study, a high-protein diet led to deterioration of glucose metabolism, but lowered BW in *db* mice under pair-fed conditions for 10 weeks [12]. Therefore, we now investigated how diets with varying protein and carbohydrate contents may affect the diabetic condition of *db* mice for a shorter duration without also affecting BW. The *db* mice fed a high-protein, low-carbohydrate diet showed significantly higher values of water intake, urinary volume, and urinary glucose than *db* mice fed a low-protein, high-carbohydrate diet, but did not exhibit a significant BW change. These mice experienced worsening diabetic symptoms associated with insufficient insulin secretion as evidenced by urinary C-peptide excretion levels, which have also been observed in human diabetic patients [17]. This finding suggests that *db* mice fail to compensate for protein-induced gluconeogenesis due to reduced capacity for insulin secretion [10].

We selected *db* mice because these mutants exhibit relatively low insulin secretion but enhanced gluconeogenesis and are commonly used as a model for studying type 2

diabetes [10, 11]. Similarly, using a type 1 diabetes animal model with insufficient insulin secretion due to β-cell damage, Linn et al. [18] reported that a high-protein diet deteriorated the animals' diabetic condition. In that study, animals fed a diet composed of 38 % protein, 19 % carbohydrate, and 43 % fat experienced a significantly higher incidence of diabetes and reduced β-cell mass than animals fed a diet of 17 % protein, 43 % carbohydrate, and 39 % fat. Based on previous and current studies, insufficient insulin secretion may be related to the deterioration of glucose metabolism induced by a high-protein diet, although these effects could be ameliorated if insulin secretion can be restored. Earlier studies indicate that Asian populations tend to show a lower capacity for insulin secretion than Caucasian populations [9, 10]. Thus, Asian patients may be more sensitive to dietary protein than Caucasian people with respect to DM development, due to their lower insulin secretion capability.

Data related to glucose metabolism from mice kept in metabolic cages were not consistent with data obtained from mice at the end of the experimental period. In our previous experiments, *db–H* mice ate more food than *db–L* mice under ad libitum conditions [12]. The present experiment was performed using pair-fed conditions; therefore, the *db–H* mice may have fasted longer than the *db–L* mice. The data on urinary glucose obtained from mice in metabolic cages may correspond precisely to the daily glucose metabolism of *db* mice.

Although dietary protein impacted several renal manifestations, including kidney weight, Ccr, and urinary albumin, *db* mice had significantly lower weight of kidneys than CT mice fed with the same diet. The high-protein effects are consistent with previous reports [12, 19, 20], but the lower-weight kidneys of the *db* mice are inconsistent with findings of some studies [12, 20]. This inconsistency could be explained by the age at which the mice were killed, which was at 6 weeks of age in our study, likely before the onset of many diabetic complications. Additionally, increased urinary C-peptide excretion may be linked to lower kidney weight under diabetic conditions [21]. Recently, Nordquist et al. [21] reported that urinary C-peptide can suppress glomerular filtration rate in mice through constriction of glomerular afferent arteries, resulting in lower-weight kidneys, which our data support. Our results also indicate that a high-protein diet increased urinary albumin in both CT and *db* mice, which is inconsistent with our previous report regarding *db* mice under long-term conditions [12]. Although further experiments are needed to determine the direct effect of dietary protein on renal manifestations, this discrepancy may be explained by the presence or absence of BW change, as BW has been associated with urinary albumin concentration [22]. However, our current study utilized short-term experiments in which we observed no significant BW change; therefore, the effects of a high-protein diet may be more clearly reflected in urinary albumin excretion levels.

To specifically investigate how dietary protein affects glucose metabolism, we selected diets composed of various amounts of proline, maltose or soluble starch, and linoleic acid. Proline is a common amino acid found in many foods of the Western diet (e.g., white bread and cheese) and the Japanese diet (rice and momen-tofu) (Table 3). As shown in Table 3, proline may be consumed at higher levels in Western countries, based on the high ratios of proline in Western versus Japanese foods. Proline is also the second most plentiful amino acid in casein and soy bean proteins, which were used as the protein sources in the diet experiment [23].

Moreover, proline is a highly soluble amino acid [24] and favourable substrate for gluconeogenesis [25]. The composition of amino acids in the diet greatly influences the biological value of protein, and physiological processes like gluconeogenesis might therefore be altered by diets containing a single type of amino acid. Therefore, the results of the present study should be considered carefully. We selected linoleic acid because it has the highest fatty acid content in soybean oil [26]. As shown in Fig. 2, the difference was small between mice treated with the *db–L* mixture and *db–H* mixture. However, the amount administered (0.28 kcal/25 g of BW) was 1/50th, compared to the diet consumed in a day (13.9 kcal) (Table 1 and supplementary Table 1). Therefore, this significant difference could explain the difference in the diet experiments. We found that *db–L* mixture mice had higher glucose levels at 30 min post-administration with a maltose-based mixture than *db–H* mixture mice administered with the same carbohydrates. In contrast, both *db–L* mixture mice and *db–H* mixture mice administered a soluble starch mixture showed similar glucose levels at 30 min post-administration. During the later phases of nutrient uptake, the *db–H* mixture mice administered the starch mixture showed significantly higher glucose levels than the starch-administered *db–L* mixture mice, indicating that higher glucose levels were not relieved by increased insulin levels, possibly due to higher insulin resistance [27, 28]. Additionally, blood glucose levels were similar from 120 to 300 min in the *db* mice treated with the two maltose mixtures. The postprandial analyses of glucose and insulin after the intake of mixtures containing either maltose or starch revealed different results depending on the source and amount of carbohydrate. Although the diets used in the metabolic cage experiments were composed of α and β cornstarch and sucrose, the difference was in the amount of α cornstarch, which resembled the soluble starch used in the postprandial analysis. In addition to the diet experiments, the postprandial analyses showed higher glucose levels but similar insulin levels in

Table 3 Composition of amino acids of staple food and representative side dishes in Japan and Western countries

Amino acid	Staple foods						Side dishes									
	Well-milled rice		White table bread		Ratio 1	Difference 1	Momen-tofu		Beef		Cheese		Ratio 2	Difference 2	Ratio 3	Difference 3
	mg	%	mg	%		mg	mg	%	mg	%	mg	%		mg		
Alanine	130	5.6	260	3.0	2.00	130	300	4.1	1100	6.1	670	2.7	3.67	800	2.23	370
Arginine	190	8.2	290	3.4	1.53	100	560	7.7	1200	6.6	820	3.4	2.14	640	1.46	260
Aspartic acid	220	9.5	350	4.0	1.59	130	850	11.7	1700	9.4	1700	7.0	2.00	850	2.00	850
Cystine	53	2.3	190	2.2	3.58	137	95	1.3	210	1.2	120	0.5	2.21	115	1.26	25
Glutamic acid + glutamine	410	17.8	3000	34.7	7.32	2590	1400	19.3	2800	15.5	5000	20.5	2.00	1400	3.57	3600
Glycine	110	4.8	320	3.7	2.91	210	300	4.1	1100	6.1	440	1.8	3.67	800	1.47	140
Histidine	61	2.6	200	2.3	3.28	139	190	2.6	730	4.0	720	3.0	3.84	540	3.79	530
Isoleucine	91	3.9	310	3.6	3.41	219	320	4.4	810	4.5	1200	4.9	2.53	490	3.75	880
Leucine	190	8.2	600	6.9	3.16	410	560	7.7	1500	8.3	2300	9.4	2.68	940	4.11	1740
Lysine	83	3.6	170	2.0	2.05	87	450	6.2	1600	8.8	1900	7.8	3.56	1150	4.22	1450
Methionine	61	2.6	130	1.5	2.13	69	92	1.3	520	2.9	580	2.4	5.65	428	6.30	488
Phenylalanine	120	5.2	440	5.1	3.67	320	380	5.2	740	4.1	1200	4.9	1.95	360	3.16	820
Proline	*110*	*4.8*	*1000*	*11.6*	*9.09*	*890*	*390*	*5.4*	*840*	*4.6*	*2600*	*10.7*	*2.15*	*450*	*6.67*	*2210*
Serine	130	5.6	410	4.7	3.15	280	380	5.2	720	4.0	1100	4.5	1.89	340	2.89	720
Threonine	84	3.6	240	2.8	2.86	156	280	3.9	820	4.5	830	3.4	2.93	540	2.96	550
Tryptophan	35	1.5	94	1.1	2.69	59	98	1.4	210	1.2	290	1.2	2.14	112	2.96	192
Tyrosine	96	4.2	280	3.2	2.92	184	280	3.9	620	3.4	1300	5.3	2.21	340	4.64	1020
Valine	130	5.6	360	4.2	2.77	230	330	4.5	890	4.9	1600	6.6	2.70	560	4.85	1270
Total	2304	100.0	8644	100.0	3.75	6340	7255	100.0	18,110	100.0	24,370	100.0	2.50	10,855	3.36	17,115

The values of the respective amino acids are shown as weight (mg) and percentage (%) per 100 g foods. The values were calculated from the data published by The Ministry of Education, Culture, Sports, Science, and Technology in Japan [32]

Ratio 1 and Difference 1 mean the ratio and difference, respectively, of weight of amino acid of well-milled rice and white table bread

Ratio 2 and Difference 2 mean the ratio and difference, respectively, of weight of amino acid of momen-tofu and beef

Ratio 3 and Difference 3 mean the ratio and difference, respectively, of weight of amino acid of momen-tofu and cheese

Proline is stressed by italic

the *db* mice, but not in the control mice, indicating that by using the high-proline mixture containing starch, the higher blood glucose could not be compensated by higher insulin secretion, which was similar to the high-protein diet experiments with the metabolic cage. The higher blood glucose in *db* mice administered the high-proline mixture containing starch could be explained by the different content of starch. However, the different amount of proline might affect the results; therefore, further studies are required to determine how different amounts and sources of proteins and carbohydrates are involved in glucose and insulin metabolism.

This result is consistent with a previous report on type 1 diabetes in human patients [29] that found subjects required more insulin after ingesting a high-protein diet, indicating insufficient insulin response and increased insulin resistance [29]. Depending on an individual's insulin secretion capacity, a high-protein diet may also deteriorate glucose metabolism in patients with type 2 diabetes. Of note, the composition of diets is not precisely known in terms of chemical substances; therefore, known chemical substances are necessary to confirm the effects of a high-protein diet. In our present study, the results obtained in the diet experiment were reproduced with the administration of known chemical substances, including maltose or soluble starch, proline, and linoleic acid (Figs. 1, 2) [12]. To examine the effect of high protein, this result obtained from the experiments with pure chemical substances has been thought to be significant.

Dietary protein also affects intestinal carbohydrate absorption. For example, a low-protein diet has a relatively large amount of polysaccharides, which normally suppress the intestinal absorption of glucose [30, 31]. Our present study suggests that when starch-based carbohydrates such as polysaccharides are present (i.e., as found in Asian foods), a low-protein diet may be recommended to Asian diabetic patients more readily than to the Caucasian patients, due to the consideration of insulin secretion capacity.

In conclusion, high-protein and low-carbohydrate diet deteriorated glucose metabolism in addition to showing renal manifestations, including urinary albumin in association with insufficient insulin secretion. Although these findings were obtained via animal experiments, the various insulin secretion capacities of different populations of humans should be considered when patients with DM are treated by dietary manipulation.

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Author contributions E.A., W.P., and M.H. obtained and analysed data and wrote the manuscript. A.M., M.N., M.A., and M.U. contributed to discussions of experimental design and analysis and reviewed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

Ethical standard This study was approved by the Ethics Committee for Animal Experimentation at Kagoshima University.

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