Enzymatically-Synthesized Glycogen Induces Cecal Glucagon-Like Peptide-1 Production and Suppresses Food Intake in Mice

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Summary It is well known that dietary fiber stimulates the release of satiety hormones such as glucagon-like peptide-1 (GLP-1), which in turn suppresses appetite. In order to evaluate appetite regulating role of enzymatically synthesized glycogen (ESG, one of the resistant starch), we examined the effects of dietary supplementation of ESG on food intake and cecal proglucagon gene expression in normal and high fat diet-fed mice. Twenty four male ICR mice were weighed and assigned to four groups: normal diet group; normal diet containing 25% ESG group; high-fat diet (HFD) group; HFD containing 25% ESG group. Each group was fed the relevant diets for 3 wk. All data were analyzed by a two-way ANOVA with the main effects of HFD and ESG. ESG significantly decreased food intake and increased the weight of the cecum and cecal content. Plasma total short chain fatty acids concentration was significantly elevated by ESG. The mRNA levels of proglucagon in the cecum and plasma total GLP-1 concentration were significantly increased by ESG. The mRNA levels of appetite regulating neuropeptides such as neuropeptide Y, agouti-related protein, proopiomelanocortin, and cocomain- and amphetamine-regulating transcript in the hypothalamus were not influenced by ESG. There is no significant interaction between diet and ESG in any parameters. These results suggest that ESG-induced upregulation of GLP-1 production in the cecum suppresses food intake in mice and that fecal fermentation may be involved in the anorexigenic effect.

Key Word appetite, GLP-1, food intake, mice, HFD

Obesity is the major risk factor for several causes of diseases, including cardiovascular disease (1) and type 2 diabetes (2). The prevalence of overweight and obese people has globally increased in recent decades. For example, the number of individuals with a body mass index (BMI) of 25 kg/m² or greater increased from 857 million in 1980, to 2.1 billion in 2013 (3). Overall mortality was lowest in adults with a BMI of about 22.5–25 kg/m² in both sexes and at all ages, and above this range, each 5 kg/m² higher BMI was associated with about 30% higher all-cause mortality (1). Therefore, new drugs or food ingredients that effectively prevent obesity should be developed for pharmacotherapy or alimentotherapy for obesity-related disorders.

Obesity results from the dysregulation of energy homeostasis including increased food intake and decreased energy expenditure. Therefore, appetite regulatory system is one of the therapeutic targets in obesity treatment (4). The hypothalamus is one of the best-studied brain region involved in the regulation of food intake. Orexigenic neuropeptide Y (Npy) and agouti-related peptide (Agrp)-expressing neurons and the anorexigenic proopiomelanocortin (Pomc)-expressing neurons in the hypothalamus play crucial roles in the central regulation of food intake (4). Recent findings suggest that dietary fiber influences appetite via hypothalamic neuropeptides including Npy, Agrp, Pomc, and cocaine and amphetamine regulated transcript (Cart) (5). It is therefore possible that dietary fiber is a promising candidate for a food ingredient to prevent or ameliorate obesity.

Enzymatically synthesized glycogen (ESG) from starch using isoamylase (EC 3.2.1.68), branching enzyme (EC 2.4.1.18), and amyloglucosidase (EC 2.4.1.25) (6) showed various biological properties, including immunostimulating activity (7), indigestibility and intestinal environment improving effect (8). Moreover, the addition of ESG in a high fat diet resulted in significant decreases in total body fat mass in rats as well as visceral fat mass (9). Dietary ESG also significantly increased the amount of lipid in the feces in rats (9). The combination of dietary ESG and exercise effectively reduced body fat mass in high fat diet (HFD)-fed mice (10). Thus, ESG is the candidate for use as a food ingredient to prevent obesity in human.

There is evidence that dietary ESG also increased the amounts of cecal short chain fatty acids (SCFA) and the viable counts of fecal Bifidobacterium and Lactobacillus in rats (8). It is therefore likely that dietary ESG functions like dietary fiber. Dietary fiber-increased colonic SCFA stimulates the release of satiety hormones such as glucagon-like peptide-1 (GLP-1), which in turn suppresses appetite and energy intake (5). Lines of evidence suggest that appetite regulating neuropeptides in the hypothalamus are involved in the anorexigenic action of GLP-1 (11–13). However, the effect of ESG on the
appetite-related factors including cecal GLP-1 production, plasma GLP-1 levels, and hypothalamic appetite regulating neuropeptides have not been investigated.

In this study, we aimed to evaluate the effect of ESG on GLP-1 release and food intake in HFD-fed mice. Our results suggest that dietary ESG suppresses appetite by the induction of cecal GLP-1 production.

**MATERIALS AND METHODS**

**Animals and diets.** The animal experiment in the present study was approved by the Institutional Animal Care and Use Committee (Permission number: 28-02-01) and carried out according to the Kobe University Animal Experimentation Regulations. Male ICR mice, 5-wk-old, were purchased from Japan SLC, Inc. (Shizuoka, Japan). The mice were housed individually in plastic cages at 25˚C in a room with an automatically controlled 12 h light:dark cycle (lights on from 1500 to 0300). Upon arrival, the mice were fed a nonpurified diet (DC-8, 23.1% crude protein and 3,590 kcal/kg, CLEA Japan, Inc., Tokyo, Japan) and acclimated to the facility for 6 d before feeding of the experimental diets. The mice had free access to water and assigned diets throughout the experimental period. Feed intake was measured three times a week. Body weight was measured every week.

**Experimental design.** Twenty-four male ICR mice were weighed and assigned to four groups (n=6): normal diet group (ND); normal diet containing 25% ESG group (ND+ESG); high-fat diet group (HFD); HFD containing 25% ESG group (HFD+ESG). The composition of experimental diets was shown in Table 1. The composition of ND is the same as that of AIN93G (14), and the composition of HFD is the same as that of commercial HFD (HFD60, Oriental Yeast Co., Ltd., Tokyo, Japan). ESG was obtained from Ezaki Glico Co., Ltd. (Osaka, Japan). The dietary fiber content of glycogen was estimated to be approximately 17~22% from analyses data (15), so 20% of ESG was replaced with cellulose, and 80% was replaced with cornstarch and maltodextrin to balance the calorie in the diet in each diet group. Each group was fed the relevant diets for 3 wk. At the end of the feeding period, mice were anesthetized by inhalation of isoflurane (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) under an ad libitum feeding condition in a dark period, and their blood was collected from the abdominal vein by using a 27G needle and a 1 mL syringe. Aprotinin (500 kIU/mL of blood) and EDTA (1 mg/mL of blood) were used as a protease inhibitor and anticoagulant, respectively. Plasma was separated immediately by centrifugation at 3,000 g for 10 min at 4˚C, frozen by liquid nitrogen and stored at −80˚C for plasma components analysis. The liver, epididymal adipose tissues, perirenal adipose tissues, gastrocnemius muscles, cecum, and cecum content were weighed. The liver and perirenal adipose tissues were then immediately frozen in liquid nitrogen for storage at −80˚C before real-time PCR analysis. The diencephalon and cecum were collected and preserved in RNAlater® tissue storage reagent (Sigma-Aldrich Co., St. Louis, MO, USA). The hypothalamus was dissected from the diencephalon based on reference to a brain atlas (16).

**Plasma components analysis.** Plasma glucose, non-esterified fatty acid (NEFA), and triglyceride were measured by using commercial kits (LabAssay™ Glucose, LabAssay™ NEFA, and LabAssay™ Triglyceride, respectively, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Plasma lactic acid and SCFA were measured by using a short chain fatty acid labeling kit (XSRFAR01, YMC Co. Ltd., Kyoto, Japan) and HPLC column (YMC-Pack FA, YMC Co. Ltd.). Plasma insulin (LBIS® Mouse Insulin

| Table 1. Composition of the experimental diets (%).  |
|---------------------------------------------------|
| ND | ND+ESG | HFD | HFD+ESG |
|---|---|---|---|
| Casein | 20 | 20 | 25.6 | 25.6 |
| l-Cystine | 0.3 | 0.3 | 0.36 | 0.36 |
| Corn starch | 39.7486 | 24.7386 | 16 | 1.5 |
| ESG | — | 25 | — | 25 |
| Maltodextrin | 13.2 | 8.21 | 6 | 0.5 |
| Sucrose | 10 | 10 | 5.5 | 5.5 |
| Cellulose | 5 | — | 6.61 | 1.61 |
| Soybean oil | 7 | 7 | 2 | 2 |
| Lard | — | — | 33 | 33 |
| Calcium carbonate | — | — | 0.18 | 0.18 |
| Mineral mixture1 | 3.5 | 3.5 | 3.5 | 3.5 |
| Vitamin mixture2 | 1 | 1 | 1 | 1 |
| Choline bitartrate | 0.25 | 0.25 | 0.25 | 0.25 |
| tert-Butylhydroquinone | 0.0014 | 0.0014 | — | — |
| Crude protein (%) | 18 | 18 | 23 | 23 |
| Energy (kcal/100 g) | 387.7 | 387.7 | 518.2 | 518.2 |

1 AIN-93G mineral mixture (Japan SLC, Inc., Shizuoka, Japan).
2 AIN-93 vitamine mixture (Japan SLC, Inc.).
Table 2. Primer sequences for real-time PCR analysis.

| Gene        | Primer sequences | Accession number |
|-------------|------------------|------------------|
| Proghucagon | sense 5′-CCA AGA GGA ACC GGA ACA AC-3′ | NM_008100 |
| Npy         | sense 5′-TCC TTC AGC ATG CCT CTC AA-3′ | NM_023456 |
| Agrp        | sense 5′-CAG AGG TGC TAG ATC CAC AGA-3′ | NM_001271806 |
| Pomp        | sense 5′-CTC CTG CTT CAG ACC TCA ATA GA-3′ | NM_001278581 |
| Cart        | sense 5′-AGC TGA TCG AAG GTG TGC A-3′ | NM_001081493 |
| Gpl         | sense 5′-TCC TGC TGC TGC ATG A-3′ | NM_131998 |
| Gs2         | sense 5′-CCA AGC AGC GAT GCC TTT AA-3′ | NM_145572 |
| Pc          | sense 5′-AGC AGG ACA CAG GGC AGA TG-3′ | NM_001162946 |
| Pdk4        | sense 5′-CCC AAA CTG TGA TGT GGT AGC A-3′ | NM_013743 |
| Pfk1        | sense 5′-CCA CAG CTG CTG CAG AAC AC-3′ | NM_011044 |
| Acca        | sense 5′-GCC TCA GGT CAC CAA AAA GAA T-3′ | NM_133360 |
| Fas         | sense 5′-GTC CCC GCC ACA TAA CTG AT-3′ | NM_007988 |
| Cpt1a       | sense 5′-GAA GCC TCG CAT GCC AAG A-3′ | NM_008826 |
| Rps17       | sense 5′-CCG GTG CAT CAT CCA GAA GA-3′ | NM_009092 |

1 Refer to GenBank accession number. Abbreviations: Npy, neuropeptie Y; Agrp, agouti related peptide; Pomc, proopiomelanocortin; Cart, cocaine and amphetamine regulated transcript; Gpl, glycogen phosphorylase; Gs2, glycogen synthase 2; Pc, pyruvate carboxylase; Pdk4, pyruvate dehydrogenase kinase 4; Pepck1, phosphoenolpyruvate carboxykinase 1; Pfk1, phosphofructokinase 1; Acca, acetyl-CoA carboxylase α; Fas, fatty acid synthase; Cpt1a, carnitine palmitoyltransferase 1a; Rps17, ribosomal protein S17.

Table 3. Effects of a high fat diet and enzymatically synthesized glycogen on body and tissue weights in mice.

|                      | ND     | ND+ESG | HFD   | HFD+ESG | Diet | ESG | Interaction |
|----------------------|--------|--------|-------|---------|------|-----|-------------|
| Body weight (g)      | 40.2±1.2 | 39.4±0.4 | 39.2±1.1 | 38.5±0.6 | —    | —   | —           |
| Food intake (g)      | 90.7±3.5 | 84.0±2.3 | 66.9±1.8 | 62.6±1.6 | *    | **  | —           |
| Energy intake (kcal) | 351.6±13.6 | 325.7±8.9 | 346.7±9.3 | 324.4±8.3 | —    | *   | —           |
| Liver (g)            | 2.31±0.10 | 2.18±0.10 | 1.99±0.10 | 1.92±0.10 | **   | —   | —           |
| Gastrocnemius (g)    | 0.37±0.01 | 0.35±0.01 | 0.34±0.01 | 0.34±0.01 | —    | —   | —           |
| Epididymal adipose tissue (g) | 1.20±0.11 | 1.16±0.12 | 1.31±0.06 | 1.36±0.20 | —    | —   | —           |
| Perirenal adipose tissue (g) | 0.50±0.04 | 0.46±0.02 | 0.51±0.05 | 0.57±0.07 | —    | —   | —           |
| Cecum (g)            | 0.12±0.01 | 0.15±0.00 | 0.09±0.00 | 0.13±0.01 | **   | **  | —           |
| Cecal content (g)    | 0.28±0.04 | 0.47±0.04 | 0.23±0.03 | 0.29±0.03 | **   | **  | —           |

Values are means±SE of six mice in each group. *, **, and — indicate p<0.05, p<0.01, and p≥0.05, respectively.

ELISA Kit (T-type), FUJIFILM Wako Shibayagi Corporation, Osaka, Japan; glucagon (Glucagon ELISA Kit Wako, FUJIFILM Wako Shibayagi Corporation), total GLP-1 (GLP-1 ELISA Kit Wako, FUJIFILM Wako Corporation), and active GLP-1 (LB1S® GLP-1 (Active) ELISA Kit, FUJIFILM Wako Shibayagi Corporation) were measured by using commercial kits. Real time-PCR analysis. The mRNA levels of gut hormones and hypothalamic neuropeptide genes were analyzed by real time PCR analysis as described previ-
Primer sequences for real time PCR are shown in Table 2. Ribosomal protein S17 was amplified as an internal standard.

Statistical analysis. Data were analyzed by a two-way ANOVA with the main effects of diet and ESG. All statistical analyses were performed using a commercial software package (StatView version 5, SAS Institute, Cary, NC, USA, 1998).

RESULTS

As shown in Table 3, ESG significantly decreased food intake and energy intake. The weights of body, liver, abdominal adipose tissues, and gastrocnemius muscles were not influenced by dietary ESG. Food intake and liver weight were significantly decreased by HFD feeding. ESG significantly increased the weight of the cecum and cecal content, suggesting that ESG function as dietary fiber. These results clearly demonstrated that ESG suppresses food intake in mice.

As shown in Table 4, plasma concentration of glucose, NEFA, and TG were not influenced by dietary ESG. HFD significantly increased plasma NEFA concentration and decreased plasma lactic acid concentration. Plasma concentrations of propionic acid, butyric acid, and total SCFA were significantly increased by HFD. Total SCFA concentration in plasma was also significantly increased by ESG. Plasma concentration of insulin was significantly decreased by ESG. In contrast, plasma concentration of glucagon was significantly increased by ESG. Thus, ESG decreased insulin/glucagon ratio in plasma.

The mRNA level of proglucagon in the cecum and plasma total GLP-1 concentration were significantly increased by ESG, whereas plasma active GLP-1 concentration was not influenced by ESG (Fig. 1). The mRNA levels of appetite regulating neuropeptides in the hypothalamus were not influenced by ESG (Fig. 2). These findings suggest that ESG induced cecal GLP-1 production but did not influence the production of appetite regulatory neuropeptides in the hypothalamus.

Figure 3 shows the effects of HFD and ESG on the mRNA levels of carbohydrates and lipid metabolism related genes in the liver. Dietary ESG significantly

Table 4. Effects of a high fat diet and enzymatically synthesized glycogen on plasma parameters in mice.

| Parameter                        | ND    | ND+ESG | HFD   | HFD+ESG | Diet | ESG | Interaction |
|----------------------------------|-------|--------|-------|---------|------|-----|-------------|
| Glucose (mg/dL)                  | 207±8 | 195±12 | 184±5 | 202±6   | —    | —   | —           |
| Non-esterified fatty acid (mEq/dL)| 0.39±0.04 | 0.34±0.04 | 0.57±0.06 | 0.68±0.11 | **   | —   | —           |
| Triglyceride (mg/dL)             | 176±20| 173±31 | 119±11| 179±37  | —    | —   | —           |
| Lactic acid (mM)                 | 5.33±0.32| 5.24±0.54| 2.98±0.20| 3.60±0.21| **   | —   | —           |
| Acetic acid (μM)                 | 84.5±1.6| 86.5±1.2| 58.2±0.8| 91.2±10.1| —    | —   | —           |
| Propionic acid (μM)              | 23.7±2.2| 36.0±2.9| 70.1±9.5| 80.5±11.8| **   | —   | —           |
| Butiric acid (μM)                | 30.7±7.5| 31.0±5.4| 41.3±4.0| 54.4±6.0  | **   | —   | —           |
| Total short chain fatty acid (μM)| 139±15| 153±12 | 170±8 | 226±22  | **   | *   | —           |
| Insulin (pmol/L)                 | 607±171| 320±56 | 366±114| 171±44  | —    | *   | —           |
| Glucagon (pmol/L)                | 15.1±2.9| 16.0±2.0| 8.1±1.7| 10.7±2.1 | —    | *   | —           |
| Insulin/glucagon ratio           | 41.1±8.4| 21.7±4.4| 52.3±14.0| 15.7±4.3 | —    | **  | —           |

Values are means±SE of six mice in each group. *, **, and — indicate p<0.05, p<0.01, and p≥0.05, respectively.
increased the mRNA levels of glycogen phosphorylase (Gpl). Dietary ESG also significantly increased the mRNA levels of pyruvate carboxylase (Pc), phosphoenolpyruvate carboxykinase 1 (Pepck1), and pyruvate dehydrogenase kinase 4 (Pdk4) in the liver, suggesting that ESG stimulates gluconeogenesis. On the other hand, dietary ESG did not influence gene expression of lipid metabolism-related genes in the liver and adipose tissue. HFD significantly decreased lipogenic genes, such as acetyl-CoA carboxylase α (Accα) and fatty acid synthase (Fas), and significantly increased a lipolytic gene, such as carnitine palmitoyltransferase 1α (Cpt1a), in the liver. These results suggest that HFD suppressed de novo lipogenesis and induced fatty acid oxidation in mice.

**DISCUSSION**

Dietary fiber is fermented by resident microbiota, elevating the production of SCFA (5). Dietary ESG significantly increased cecal SCFA in rats (8). SCFA could stimulate hormonal signals that would exert an anorectic effect (5). For example, SCFA significantly increased the mRNA levels of proglucagon in vitro (17, 18). In the present study, ESG increased the mRNA levels of proglucagon in the cecum and total GLP-1 and SCFA levels in plasma. In addition, ESG significantly decreased food intake. All these findings suggest that ESG-increased the production of satiety signal GLP-1, which in turn, results decrease of food intake.

Lines of evidence suggest that GLP-1 directly influences the expression of appetite regulating neuropeptide Y (Npy), agouti related peptide (Agrp), proopiomelanocortin (Pomc), and cocaine and amphetamine regulated transcript (Cart). In the present study, ESG significantly increased the mRNA levels of hypothalamic neuropeptides in mice. Values are means±SE of six mice in each group. * and — indicate p<0.05 and ≥0.05, respectively. Abbreviations: Npy, neuropeptide Y; Agrp, agouti related peptide; Pomc, proopiomelanocortin; Cart, cocaine and amphetamine regulated transcript.
needed or conserve it when not needed (26). Hepatic glucose production by insulin is partly explained by suppression of glucagon secretion (27). All these findings suggest that ESG-induced upregulation of the mRNA levels of gluconeogenic genes in the liver is due to the changes of plasma insulin and glucagon levels.

Effects of food ingredients can be affected by the composition of experimental diets. For example, soy protein suppressed serum TG concentration in normal diet-fed rats, but not in high cholesterol diet-fed rats (28). Soy protein prevented adipocyte hypertrophy in high-fat diet-fed rats, but not in normal diet-fed rats (29). In the present study, there was no significant interaction between diet and ESG in any parameters. Our findings suggest that the effects of ESG may not be influenced by the dietary fat level in mice.

In conclusion, dietary ESG induced the expression of proglucagon in the cecum, which in turn results suppression of food intake in mice.

**Fig. 3.** Effects of enzymatically synthesized glycogen and high fat diet on the mRNA levels of carbohydrate and lipid metabolism-related genes in the liver in mice. Values are means±SE of six mice in each group. *, **, and — indicates p<0.05, p<0.01, and p≥0.05, respectively. Abbreviations: Gpl, glycogen phosphorylase; Gs2, glycogen synthase 2; Pc, pyruvate carboxylase; Pfk1, phosphofructokinase 1; Pdk4, pyruvate dehydrogenase kinase 4; Pepck1, phosphoenolpyruvate carboxykinase 1; Acca, acetyl-CoA carboxylase α; Fas, fatty acid synthase; Cpt1a, carnitine palmitoyltransferase 1a.

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**Authorship**

Research conception and design: KH; experiments: KH and AY; statistical analysis of the data: KH and AY; interpretation of the data: KH, TS, and HK; writing of the manuscript: KH, TS, and HK.

**Disclosure of state of COI**

No conflicts of interest to be declared.

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2: 183–189.
9) Furuyashiki T, Ogawa R, Nakayama Y, Honda K, Kaminosyama H, Takata H, Yasuda M, Kuriki T, Ashida H. 2013. Enzymatically synthesized glycogen reduces lipid accumulation in diet-induced obese rats. *Nutr Res* **33**: 743–752.
10) Tamura S, Honda K, Morinaga R, Saneyasu T, Kaminosyama H. 2017. Effects of enzymatically synthesized glycogen and exercise on abdominal fat accumulation in high-fat diet-fed mice. *J Nutr Sci Vitaminol* **63**: 405–411.

REFERENCES

1) Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qiu L, Collins R, Peto R. 2009. Body-mass index and cause-specific mortality in 900000 adults: collaborative analyses of 57 prospective studies. *Lancet* **373**: 1083–1096.
2) Abdullah A, Peeters A, de Courten M, Stoelwinder J. 2010. The magnitude of association between over-weight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies. *Diabetes Res Clin Pract* **89**: 309–319.
3) Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Les Maye S,勇 H, van de Vijver S, Vasankari TJ, Veerman JL, Mathers C, Ezzati M, Lopez AD, Murray CJ, Gakidou E. 2013. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study. *Lancet* **384**: 766–781.
4) Timper K, Brüning JC. 2017. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech* **10**: 679–689.
5) Chambers ES, Morrison DJ, Frost G. 2015. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proc Nutr Soc* **74**: 328–336.
6) Kajitaka H, Kakutani R, Akiyama T, Takata H, Kuriki T. 2008. A novel enzymatic process for glycogen production. *Biocatal Biotransform* **26**: 133–140.
7) Kakutani R, Adachi Y, Kajitaka H, Takata H, Kuriki T, Ohno N. 2007. Relationship between structure and immunostimulating activity of enzymatically synthesized glycogen. *Carbohydr Res* **342**: 2371–2379.
8) Furuyashiki T, Takata H, Kojima I, Kuriki T, Fukuda I, Ashida H. 2011. Metabolic fate of orally administered enzymatically synthesized glycogen in rats. *Food Funct* **2**: 183–189.
9) Furuyashiki T, Ogawa R, Nakayama Y, Honda K, Kaminosyama H, Takata H, Yasuda M, Kuriki T, Ashida H. 2013. Enzymatically synthesized glycogen reduces lipid accumulation in diet-induced obese rats. *Nutr Res* **33**: 743–752.
10) Tamura S, Honda K, Morinaga R, Saneyasu T, Kaminosyama H. 2017. Effects of enzymatically synthesized glycogen and exercise on abdominal fat accumulation in high-fat diet-fed mice. *J Nutr Sci Vitaminol* **63**: 405–411.
2018. Glucagon revisited: Coordinated actions on the liver and kidney. *Diabetes Res Clin Pract* **146**: 119–129.

25) Seitz HJ, Müller MJ, Nordmeyer P, Krone W, Tarnowski W. 1976. Concentration of cyclic AMP in rat liver as a function of the insulin/glucagon ratio in blood under standardized physiological conditions. *Endocrinology* **99**: 1313–1318.

26) Kalra S, Gupta Y. 2016. The insulin:glucagon ratio and the choice of glucose-lowering drugs. *Diabetes Ther* **7**: 1–9.

27) Lewis GF, Carpentier AC, Pereira S, Hahn M, Giacca A. 2021. Direct and indirect control of hepatic glucose production by insulin. *Cell Metab* **33**: 709–720.

28) Arellano-Martínez GL, Granados O, Palacios-González B, Torres N, Medina-Vera I, Tovar AR. 2014. Soya protein stimulates bile acid excretion by the liver and intestine through direct and indirect pathways influenced by the presence of dietary cholesterol. *Br J Nutr* **111**: 2059–2066.

29) Frigolet ME, Torres N, Uribe-Figueroa L, Rangel C, Jimenez-Sanchez G, Tovar AR. 2011. White adipose tissue genome wide-expression profiling and adipocyte metabolic functions after soy protein consumption in rats. *J Nutr Biochem* **22**: 118–129.