International Journal of Innovative Technologies in Social Science

e-ISSN: 2544-9435

Scholarly Publisher
RS Global Sp. z O.O.
ISNI: 0000 0004 8495 2390

Dolna 17, Warsaw,
Poland 00-773
+48 226 0 227 03
editorial_office@rsglobal.pl

ARTICLE TITLE	THE IMPACT OF HIGH-PROTEIN DIET ON GUT AND METABOLIC HORMONES: A SYSTEMATIC LITERATURE REVIEW 2021-2025
DOI	https://doi.org/10.31435/ijitss.4(48).2025.4403
RECEIVED	21 October 2025
ACCEPTED	22 December 2025
PUBLISHED	24 December 2025
LICENSE	The article is licensed under a **Creative Commons Attribution 4.0 International License.**

THE IMPACT OF HIGH-PROTEIN DIET ON GUT AND METABOLIC HORMONES: A SYSTEMATIC LITERATURE REVIEW 2021-2025

Karolina Swierk *(Corresponding Author, Email: kara_803@wp.pl)*
Medical University of Lodz, Lodz, Poland
ORCID ID: 0009-0003-3784-9630

Damian Podkoscielny
Medical University of Lodz, Lodz, Poland
ORCID ID: 0009-0007-4979-1674

Wojciech Machulski
Military Institute of Medicine, Warsaw, Poland
ORCID ID: 0009-0007-6337-5909

Martyna Ciarkowska
Medical University of Lodz, Lodz, Poland
ORCID ID: 0009-0001-4635-378X

Jan Makulski
1st Military Clinical Hospital with Outpatient Clinic, Lublin, Poland
ORCID ID: 0009-0006-7545-499X

Kamil Franczyk
10th Military Hospital with Outpatient Clinic, Independent Public Health Care Institution, Bydgoszcz, Poland
ORCID ID: 0009-0001-6294-5101

Maria Gierasimiuk
116th Military Hospital with Outpatient Clinic, Independent Public Health Care Institution, Opole, Poland
ORCID ID: 0009-0009-1450-9258

Michal Gorski
116th Military Hospital with Outpatient Clinic, Independent Public Health Care Institution, Opole, Poland
ORCID ID: 0009-0006-8762-2823

Adam Januszkiewicz
107th Military Hospital with Outpatient Clinic, Independent Public Health Care Institution, Wałcz, Poland
ORCID ID: 0009-0008-9488-2273

Wiktoria Januszkiewicz
107th Military Hospital with Outpatient Clinic, Independent Public Health Care Institution, Wałcz, Poland
ORCID ID: 0009-0005-5730-4333

ABSTRACT

Objective: This systematic literature review evaluates the impact of high-protein diets on key gut and metabolic hormones in humans, emphasizing GLP-1, glucagon, insulin, ghrelin, and leptin.
Methods: A systematic review of scientific literature from 2021-2025 was conducted. Randomized controlled trials (RCTs), crossover studies, and meta-analyses examining effects of high-protein diets (≥25-30% energy from protein) on hormonal and metabolic parameters were analyzed in healthy individuals and those with obesity, type 2 diabetes, and other metabolic conditions.
Results: High-protein diets consistently stimulated GLP-1 secretion, with active GLP-1 increasing 87-156% after a single meal compared to controls. Whey protein demonstrated strong insulinotropic properties, with optimal effects at 15-55g consumed 15-30 minutes pre-meal. Significant postprandial glycemia reduction was observed (mean -1.4 mmol/L; up to -2.0 mmol/L in T2DM individuals) alongside improved insulin sensitivity indices (HOMA-IR, Matsuda index). Increased glucagon/insulin ratio promoted fat oxidation and preferential adipose tissue loss while preserving lean mass. High-protein diets suppressed ghrelin, modulated leptin, and enhanced satiety, though appetite hormone effects were more complex than incretin responses. Long-term interventions (6-12 months) demonstrated visceral and hepatic fat reduction (mean -42% IHL), improved lipid profiles, and beneficial gut microbiota changes.
Conclusions: High-protein diets beneficially impact gut and metabolic hormones through incretin stimulation, glucagon/insulin ratio modulation, improved insulin sensitivity, and appetite hormone regulation. These effects translate to clinically significant metabolic improvements, particularly in individuals with type 2 diabetes and obesity. Optimal dosing (25-35% energy from protein, 1.2-2.0 g/kg/day) and timing (preload 15-30 minutes pre-meal) maximize metabolic benefits, indicating potential for effective dietary intervention in preventing and treating metabolic disorders.

KEYWORDS

High-Protein Diet, GLP-1, Glucagon, Insulin, Appetite Hormones, Metabolism

CITATION

Karolina Swierk, Damian Podkoscielny, Wojciech Machulski, Martyna Ciarkowska, Jan Makulski, Kamil Franczyk, Maria Gierasimiuk, Michal Gorski, Adam Januszkiewicz, Wiktoria Januszkiewicz. (2025) The Impact of High-Protein Diet on Gut and Metabolic Hormones: A Systematic Literature Review 2021–2025. *International Journal of Innovative Technologies in Social Science*. 4(48). doi: 10.31435/ijitss.4(48).2025.4403

1. Introduction

The epidemic of obesity and type 2 diabetes represents one of the most serious public health problems of the 21st century, affecting hundreds of millions of people worldwide. Metabolic disorders associated with excessive body weight lead to the development of insulin resistance, carbohydrate metabolism disorders, and increased risk of cardiovascular diseases. In the face of the growing prevalence of metabolic diseases, the search for effective dietary interventions constitutes a priority both in prevention and in the therapy of these conditions.

High-protein diets, defined as diets containing ≥25-30% energy derived from protein, have gained significant scientific attention as a potential strategy in body weight management and metabolic parameters. In contrast to conventional dietary recommendations, which suggest moderate protein intake (10-15% energy), high-protein diets offer a range of unique metabolic benefits that extend beyond simple caloric restriction. The mechanisms of these benefits are complex and multifaceted, encompassing modulation of key hormones regulating metabolism and appetite.

Gut hormones constitute a fundamental element in the regulation of the organism's energy homeostasis. GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide), referred to as incretins, are secreted by enteroendocrine cells in response to food intake and play a crucial role in the regulation of postprandial glycemia through stimulation of insulin secretion in a glucose-dependent manner. GLP-1 additionally inhibits glucagon secretion, delays gastric emptying, and modulates the feeling of satiety through action on the central nervous system. Growing clinical interest in incretins has been reflected in the

development of GLP-1 receptor agonist drugs, which have become a breakthrough in the therapy of type 2 diabetes and obesity.

An equally important role in metabolic regulation is played by appetite hormones, including ghrelin and leptin. Ghrelin, produced mainly in the stomach, is a key orexigenic hormone that stimulates appetite and food intake. Its levels rise before meals and fall after food consumption. In turn, leptin, secreted by adipocytes, signals the organism's energy state to the central nervous system, promoting the feeling of satiety and decreasing food intake. Paradoxically, obesity is characterized by high leptin levels and the development of leptin resistance, which disrupts the physiological regulation of appetite.

Dietary protein exerts a unique impact on the hormonal system compared to other macronutrients. It has the highest thermic effect (20-30% of consumed energy vs. 5-10% for carbohydrates and 0-3% for fats), which contributes to increased energy expenditure. Moreover, amino acids derived from dietary protein directly stimulate enteroendocrine cells to secrete GLP-1 through receptor mechanisms involving taste receptors (T1R1/T1R3), calcium-sensing receptors (CaSR), and peptide transporters (PepT1). Different protein sources, particularly whey protein, demonstrate differentiated ability to stimulate incretin and insulin response.

Despite the growing number of studies on the effects of high-protein diets, comprehensive understanding of their impact on a broad spectrum of gut and metabolic hormones requires systematic synthesis of current literature. In recent years (2021-2025), a series of new randomized controlled trials, meta-analyses, and mechanistic studies have been published that have provided new data concerning acute and chronic effects, optimal dosing and timing of protein consumption, differences between protein sources, and specific effects in clinical populations.

Objective: The aim of this systematic review is a comprehensive evaluation of the impact of high-protein diets on key gut hormones (GLP-1, GIP, ghrelin, PYY, CCK) and metabolic hormones (insulin, glucagon, leptin, adiponectin) in light of the latest scientific evidence from 2021-2025. The work aims to identify mechanisms of action of dietary protein, determine optimal dosing and timing strategies, evaluate effects in different clinical populations (type 2 diabetes, obesity, prediabetic states), and indicate directions for future research and clinical applications.

2. Methodology

2.1 Search Strategy

A systematic review of scientific literature published in 2021-2025 was conducted. Main biomedical databases were searched, including PubMed/MEDLINE, Scopus, Web of Science, and Cochrane Library. A search strategy based on a combination of MeSH terms and keywords related to high-protein diets and metabolic hormones was applied.

Applied search terms included:

- **Diets:** "high-protein diet", "protein supplementation", "whey protein", "dietary protein", "protein intake", "high-protein meal"
- **Gut hormones:** "GLP-1", "glucagon-like peptide-1", "GIP", "glucose-dependent insulinotropic polypeptide", "incretins", "gut hormones", "PYY", "ghrelin", "CCK", "cholecystokinin"
- **Metabolic hormones:** "insulin", "glucagon", "leptin", "adiponectin"
- **Metabolic parameters:** "glycemic control", "insulin sensitivity", "HOMA-IR", "postprandial glucose", "appetite regulation", "satiety"

Terms were combined using Boolean operators (AND, OR) to maximize search sensitivity and specificity.

2.2 Inclusion Criteria

Studies meeting the following criteria were included in the review:

1. **Type of studies:** Randomized controlled trials (RCTs), crossover studies, prospective cohort studies, meta-analyses, and systematic reviews concerning the impact of high-protein diets on gut and metabolic hormones.
2. **Population:** Studies conducted on adult humans (≥18 years), including healthy individuals, those with obesity (BMI ≥30 kg/m²), overweight (BMI 25-29.9 kg/m²), type 2 diabetes, prediabetic state, or other metabolic disorders.
3. **Intervention:** High-protein diets defined as:

- Protein intake ≥25-30% of total daily energy, or

- Protein intake ≥1.2-2.0 g/kg body weight/day, or
- Protein supplementation (20-70g per portion), or
- Protein preload protocols (10-55g protein consumed before main meal)

4. **Endpoints:** Studies evaluating at least one of the following parameters:

- Incretin hormones: GLP-1, GIP
- Pancreatic hormones: insulin, glucagon, glucagon/insulin ratio
- Appetite hormones: ghrelin, leptin, PYY, CCK
- Adipokines: adiponectin
- Glycemic parameters: fasting and postprandial glucose, HbA1c, HOMA-IR, insulin sensitivity indices
- Subjective appetite sensations: hunger, satiety (VAS)

5. **Publication language:** Articles published in English.
6. **Publication period:** Studies published between 2021 and 2025.

2.3 Exclusion Criteria

The review excluded:

- Animal studies (except selected mechanistic studies relevant to understanding molecular mechanisms)
- Studies conducted on children and adolescents (<18 years)
- Conference abstracts without full text
- Case reports and case series
- Studies not evaluating hormones as endpoints
- Studies concerning exclusively supplementation of individual amino acids without the context of high-protein diet
- Publications in languages other than English

2.4 Data Synthesis

Due to the heterogeneity of included studies (different intervention protocols, populations, endpoints), a narrative synthesis of results was conducted. Data were grouped thematically according to:

1. Effects on individual hormones (GLP-1, insulin, glucagon, ghrelin, leptin)
2. Acute effects (single meal) vs. chronic effects (long-term interventions)
3. Protein source (whey, plant-based, animal)
4. Clinical populations (healthy individuals, with type 2 diabetes, obesity)
5. Dosing and timing of protein consumption

For studies reporting similar endpoints, the range of values and direction of effects were presented. When meta-analyses were available, pooled effect sizes were reported with priority.

2.5 Methodological Limitations

The review is narrative in nature due to significant methodological heterogeneity of included studies. Differences in intervention protocols, definitions of high-protein diets, methods of hormone measurement, and population characteristics made it impossible to conduct quantitative meta-analysis. Formal assessment of publication bias was not performed due to the nature of narrative synthesis.

3. Results

3.1. Effects of High-Protein Diets on GLP-1

3.1.1 Acute Effects (Single Meal)

Studies have consistently demonstrated that dietary protein exerts a strong stimulatory effect on GLP-1 secretion. Smedegaard et al. (2023) conducted a meta-analysis of 27 studies and showed that whey protein preload (4-55 g) significantly reduced postprandial glucose peak (mean -1.4 mmol/L), with the effect being stronger in individuals with T2DM (mean -2.0 mmol/L). Thomas et al. (2024) compared a high-protein diabetic shake (20g protein) with oatmeal and demonstrated a 156% greater increase in active GLP-1 in the protein group (AUC 0-240min: 1826 vs 713 pg/mL x min, $p<0.001$). Simultaneously, 33% lower glucose concentration and 26% lower insulin concentration were observed, suggesting improved insulin sensitivity. In a randomized crossover study, Ekberg et al. (2024) evaluated postprandial responses in 20 individuals with type 2 diabetes and 21 individuals without diabetes after consuming four isocaloric (600 kcal) meals with

dominant content of: carbohydrates, fiber, fat, or protein. In the context of glycemic response, the high-protein meal, similarly to the high-fat meal, induced a lower incremental glycemic peak and smaller glucose iAUC compared to meals rich in carbohydrates or fiber. Lower iAUC values of the insulin:glucagon ratio (IGR) were also noted after the high-protein meal than after high-carbohydrate and high-fiber meals, indicating a more favorable metabolic profile. It should also be noted that the high-protein meal caused the highest glucagon iAUC among all tested meals, regardless of the presence of type 2 diabetes.

3.1.2 Role of Protein Source

Whey protein exhibited particularly strong insulinotropic and GLP-1 stimulating properties. Watkins et al. (2023) in their review explained the mechanisms responsible for protein-mediated GLP-1 secretion. These mechanisms include a calcium-dependent mechanism, where protein provides both amino acids and calcium, which synergistically stimulate intestinal L cells to secrete GLP-1. There is also a receptor mechanism in which amino acids activate taste receptors (T1R1/T1R3) and calcium-sensing receptors (CaSR) on enteroendocrine cells. Additionally, peptide transport via PepT1, which transports di- and tripeptides across the cell membrane, initiates a signaling cascade. Hira et al. (2021) in a study on rats showed that dietary protein accounts for the majority of postprandial GLP-1 secretion, while carbohydrates and fats play a lesser role. Sampling from the mesenteric vein of the ileum revealed that GLP-1 concentration increased proportionally to the protein content in the meal. Neacsu et al. (2021) compared different plant protein sources (hemp, buckwheat, fava bean, pea, lupin) with beef. All plant sources elicited similar or higher GLP-1 responses compared to beef, with hemp protein producing the highest GLP-1 response, while the greatest satiety (lowest hunger at 300 minutes) was noted after buckwheat.

3.1.3 Chronic Effects

In long-term studies, the glucagon response to a high-protein diet was more complex than the short-term changes observed after a single meal. In a 6-week intervention conducted by Zhang et al. (2022) in individuals with type 2 diabetes, an isocaloric high-protein diet (≈30% energy from protein) was compared with the high-carbohydrate diet used before the intervention. No significant changes in fasting glucagon concentration were noted; however, postprandial relationships between glucagon, amino acids, and hepatic fat metabolism proved crucial. The high-protein diet led to significant reduction in hepatic fat and improved insulin sensitivity, with effect size varying between participants. In the entire cohort, intrahepatic lipid content (IHL) decreased by an average of ~42% (from 15.4% to 8.8%), and in the subgroup with the greatest improvement by as much as ~65%. Insulin sensitivity indices (HOMA-IR, Matsuda, M-value) also improved, particularly in the group with greater IHL reduction (approximately ~20-30% improvement). Kempf et al. (2022) in the 12-month ACOORH study showed that early leptin reduction after 1 month was a significant predictor of long-term weight loss during a diet using high-protein low-GI meal replacement products.

3.2. Effects on Insulin and Glucagon

3.2.1 Insulin

High-protein diets showed variable effects on insulin depending on metabolic context: in healthy individuals, Oliveira et al. (2021) demonstrated that consumption of a high-protein meal replacement shake (25g protein, 230 kcal) reduced post-exercise hunger increase and increased fat oxidation, as well as increased postprandial GLP-1 compared to the control meal. Insulin after exercise increased more in the HP-MR condition rather than decreasing, reflecting a physiological response to greater incretin stimulation. In individuals with obesity, Rodrigo-Carbó et al. (2024) conducted an RCT comparing low-calorie high-protein diets based on different protein sources (pork, tuna, beef, chicken) with a control diet in 110 individuals with prediabetes or type 2 diabetes. After 6 months, all high-protein groups showed significant reduction in fasting insulin (from -16% to -24%), improvement in HOMA-IR (from -23% to -32%), and reduction in HbA1c (from -0.4% to -0.6%). Importantly, these effects were independent of protein source. In individuals with type 2 diabetes, Skytte et al. (2021) investigated the effects of carbohydrate restriction (30g/day), which naturally increased the proportion of protein and fat, in 28 patients over 6 weeks. A 53% reduction in postprandial insulin response, 44% reduction in postprandial glycemia, and improvement in pancreatic beta cell function, measured as increased glucose-corrected insulin secretion index, were observed.

3.2.2 Glucagon and Glucagon/Insulin Ratio

Glucagon, traditionally perceived as a counter-regulatory hormone to insulin, proved to be a key mediator of the beneficial metabolic effects of high-protein diets. Zhang et al. (2022) demonstrated that in individuals with type 2 diabetes, a high-protein diet (30% energy from protein) for 6 weeks improved insulin sensitivity, particularly in individuals with greater IHL reduction (up to ~30% improvement in indices). Although it did not affect fasting glucagon concentration, it altered postprandial glucagon-amino acid relationships, which after intervention strongly correlated with metabolic parameters. As a result, it improved metabolic profile, including glycemia and OGTT/MMTT parameters, without the need to increase basal glucagon secretion. Schuppelius et al. (2024) in a twin study investigated the effects of 6-week isoenergetic diets rich in fat (50%E) or protein (30%E) in healthy, lean adults. The high-protein diet caused a higher fasting glucagon/insulin ratio (+18%), greater post-meal glucagon increase (+65%), and reduction in visceral adipose tissue. The authors proposed that the increased glucagon/insulin ratio promotes fat oxidation and catabolism, which may explain the thermogenic effect of high-protein diets and preferential loss of adipose tissue. Ang et al. (2023) investigated the effect of a single 70g dose of whey protein in healthy men. They showed that consumption of a large protein dose causes a pronounced increase in glucagon concentration in postprandial response. Elevated glucagon strongly stimulates gluconeogenesis from amino acids, which constitutes a significant source of glucose appearing in the blood after a protein meal. Moreover, gluconeogenesis from amino acids is an energetically costly process, leading to increased energy expenditure after protein consumption and explaining the high thermic effect of protein. Mechanism: Amino acids, particularly arginine, leucine, and alanine, directly stimulate pancreatic alpha cells to secrete glucagon. Glucagon then activates gluconeogenesis in the liver, an energy-consuming process, which contributes to the increased thermic effect of protein.

A summary of insulin and glucagon responses across studies is presented in Table 1.

Table 1. Effects of High-Protein Diets on Insulin and Glucagon in Human Studies

Study	Population / Intervention	Main Outcomes
Rodrigo-Carbó et al., 2024	110 individuals with obesity; 4 different high-protein diets	↓ Fasting insulin (−16% to −24%); ↓ HOMA-IR (−23% to −32%); ↓ HbA1c (−0.4 to −0.6%)
Skytte et al., 2021	28 individuals with T2DM; carbohydrate restriction (↑ protein proportion)	↓ Postprandial insulin −53%; ↓ glycemia −44%; ↑ β-cell function
Schuppelius et al., 2024	Healthy lean adults; 6-week 30%E protein diet	↑ Glucagon/insulin ratio +18%; ↑ post-meal glucagon +65%; ↓ visceral fat
Ang et al., 2023	Healthy men; 70 g whey protein challenge	Strong ↑ glucagon; ↑ gluconeogenesis; ↑ energy expenditure (thermic effect)

3.3. Effects on Appetite Hormones: Ghrelin and Leptin

3.3.1 Ghrelin (Hunger Hormone)

Ghrelin, produced primarily in the stomach, is a key orexigenic hormone (appetite-stimulating). High-protein diets consistently demonstrated the ability to suppress ghrelin. Deru et al. (2023) investigated the effect of exercise during a 36-hour fast in healthy adults. Performing exercise at the beginning of the fast decreased ghrelin concentrations compared to fasting without exercise (approximately -17% AUC, $p<0.01$) and simultaneously increased GLP-1 AUC compared to fasting without exercise. Interestingly, this did not cause significant changes in subjective feelings of hunger and appetite, despite observed changes in hormones. Although the study did not include a high-protein diet, the results illustrate the plasticity of the ghrelin system in response to the interaction between exercise and metabolic state. In the study by Ataeinosrat et al. (2022), 44 men with obesity (BMI approximately 32.9) were randomized to four training groups or control. After the intervention, in all three training groups (TRT, CRT, IRT) there was a significant decrease in ghrelin concentration (as well as leptin, PYY, cholecystokinin) compared to the control group. Additionally, GLP-1 increased significantly in the CRT and IRT groups, and adiponectin levels increased in all training groups compared to control. From this it may follow that combining exercise with a high-protein diet may therefore synergistically modulate appetite. Zhu et al. (2021) in the DIOGENES study (n=768, 34 European centers) showed that a high-protein/low-GI diet for 6 months effectively suppressed hunger in the weight maintenance

phase after weight loss (-12% vs. baseline, p<0.001), although no significant differences in fasting ghrelin levels between diets were observed. This suggests that the satietogenic effects of protein may be mediated by mechanisms other than basal ghrelin suppression.

3.3.2 Leptin (Satiety Hormone)

Leptin, produced by adipocytes, signals the body's energy state to the brain. Paradoxically, obesity is characterized by high leptin levels and resistance to its action. In a case-control study, Hajishizari et al. (2022) compared women with obesity with hypo-RMR (low resting energy expenditure) to women with normo-RMR. In women with hypo-RMR, lack of significant association between leptin levels and RMR, higher ghrelin levels, and greater leptin resistance were demonstrated. Simultaneously, weaker hunger and appetite sensations were noted, as these parameters showed positive association with RMR, suggesting that higher RMR is associated with stronger hunger. Kempf et al. (2022) in the 12-month ACOORH study demonstrated the key role of early leptin reduction as a prognostic biomarker: Individuals in the highest tertile of leptin reduction after 1 month lost the most body weight over the year (approximately 9-10kg depending on group), while individuals with the smallest leptin reduction (lowest tertile) achieved significantly less weight loss. Early leptin reduction predicted long-term weight loss independently of age, sex, and BMI (multifactorial analyses), which has clinical implication: leptin measurement after 4 weeks of intervention can identify individuals "responding" to therapy, enabling early personalization of dietary strategy. In an 8-week RCT in women with obesity, Elahikhah et al. (2024) compared milk protein concentrate (MPC, 30g/day) supplementation with placebo. The MPC group showed significantly greater leptin reduction compared to the control group (p=0.014), significant improvement in insulin resistance (HOMA-IR decrease, p=0.020), greater fat mass reduction (approximately -4.5kg based on tabular results), and significant adiponectin increase after intervention (p=0.032). Adiponectin, unlike leptin, is inversely correlated with adipose tissue and promotes insulin sensitivity, fat oxidation, and has anti-inflammatory properties. A detailed summary of the study results is presented in Table 2.

Table 2. Key Findings from Studies on Leptin and Interventions in Individuals with Obesity.

No.	Study (Authors, Year)	Population/ Study Groups	Key Finding/Result	Clinical Application/Implication
1.	Hajishizari et al. (2022)	Women with obesity (hypo-RMR vs. normo-RMR)	**Hypo-RMR:** Higher leptin resistance, higher ghrelin levels, no significant association between leptin and RMR. **Normo-RMR:** Stronger subjective hunger/appetite (positive association with RMR).	Low RMR may be associated with increased leptin resistance and altered appetite regulation.
2.	Kempf et al. (2022) - ACOORH Study	Overweight/obese individuals (12-month intervention)	**Early leptin reduction (after 1 month) is a predictor:** Individuals in the highest tertile of reduction lost the most weight (approx. 9-10 kg/year); those with the lowest reduction had the smallest weight loss.	Measuring leptin after 4 weeks of intervention can **identify treatment responders** and allow for early personalization of dietary strategies.
3.	Elahikhah et al. (2024) - RCT	Women with obesity (Milk Protein Concentrate MPC vs. placebo supplementation, 8 weeks)	**MPC Group vs. Placebo:** Significantly greater decrease in leptin (p=0.014), decrease in HOMA-IR (p=0.020), greater fat mass reduction (approx. -4.5 kg), increase in adiponectin (p=0.032).	MPC supplementation can be an **effective dietary strategy** to support weight loss, decrease insulin resistance, and improve adipokine profile (leptin, adiponectin).

3.4. Effects on Satiety and Food Intake

Modulation of gut hormones translates into subjective feelings of appetite and actual energy intake.

3.4.1 Acute Effects on Satiety

Oliveira et al. (2022) in a study on healthy individuals with normal body weight compared a 32-hour high-protein total diet replacement model (HP-TDR: 35% protein) with a control diet. The HP-TDR diet increased postprandial GLP-1 and PYY concentrations, indicating increased satiety signaling. Nevertheless, it did not change subjective feelings of hunger and satiety (VAS), which were similar between diets. Importantly, despite being isocaloric, it favorably modulated hormonal response without changes in fasting GLP-1 levels. Buso et al. (2021), analyzing data from the DiOGenes project, evaluated the impact of a high-protein low-GI diet on maintaining hormonal changes after weight loss. After randomization to the 6-month maintenance phase, it was shown that there were no significant differences between the high-protein/low-GI diet and the control diet in subjective feelings of satiety and hunger. Moreover, the high-protein/low-GI diet did not affect fasting ghrelin and PYY levels significantly differently than the control diet, and the authors did not note clear differences between diets in fasting GLP-1 levels (this parameter was not the main analyzed point in group comparison). Consequently, the authors concluded that satietogenic effects may be more related to postprandial hormone changes than basal levels. Dalgaard et al. (2023) conducted a randomized crossover study comparing a high-protein breakfast (PRO) with a low-protein breakfast (CHO) and skipping breakfast (CON). It was shown that the high-protein breakfast significantly increased postprandial satiety, reflected by greater area under the curve (AUC) for satiety, fullness, and satisfaction over 3 hours after the meal. Additionally, it reduced feelings of hunger, desire to eat, and "prospective consumption" compared to CHO and CON. Furthermore, it improved cognitive concentration, increasing the attention test score performed 150 minutes after the meal (difference ~3.5 percentage points versus CON). Despite these benefits, the high-protein breakfast did not significantly affect postprandial ghrelin, GLP-1, or CCK concentrations, and also did not change energy intake during ad libitum lunch or total daily intake.

3.5 Molecular Mechanisms and Novel Discoveries

3.5.1 Novel Hormones Responsive to Dietary Protein

Recent studies have revealed the existence of specialized gut hormones that respond to dietary protein content and regulate food choice and metabolic processes in animal models (Ahrentløv et al., 2025; Yoshinari et al., 2024). Yoshinari et al. (2024) identified CCHamide1 (CCHa1) as a high-protein-responsive gut hormone. CCHa1 is secreted by enteroendocrine cells in response to protein-rich diets or essential amino acid mixtures, and its function is to inhibit excessive protein consumption. It acts through specialized intestinal neurons producing short neuropeptide F (sNPF). Loss of CCHa1 leads to increased protein appetite, metabolic disorders (e.g., ammonia accumulation), and shortened lifespan under high-protein diet conditions. Ahrentløv et al. (2025) identified tachykinin (Tk) as a protein-responsive gut hormone. Tk is secreted by intestinal enteroendocrine cells in response to high-protein food and acts through AKH-producing cells, affecting the gut-brain axis. Its behavioral effect consists of inhibiting appetite for protein after its consumption and promoting increased appetite for sugars, enabling macronutrient balancing. The relationship with longevity is evident in that inhibition of Tk signaling extends lifespan, demonstrating the role of the gut-brain axis in controlling diet-dependent aging (AKH-dependent effect). As a result, Tk serves as a "protein satiety hormone" that counteracts excessive protein consumption.

Implications for humans: Although the results come from Drosophila, the discovery of specialized hormones responding to specific macronutrients indicates the possibility of similar mechanisms existing in mammals. In humans, analogous roles may be played by, among others, FGF21 (response to protein deficiency), NPY, substance P, or neuromedin U - all participate in diet composition-dependent appetite regulation.

3.5.2 Role of Gut Microbiota

Dugardin et al. (2022) investigated the effect of different dietary protein sources on regulation of glucose absorption in the intestine, analyzing their action at the enterocyte level and in an animal model. Key findings: Different dietary proteins differentially affect glucose transport, including through modulation of GLUT2 transporter expression in enterocytes. Digested proteins can reduce glucose absorption in the intestine, affecting postprandial glucose homeostasis. Protein sources (including animal and plant) differ in the composition of peptides arising during digestion, which translates into differences in their impact on intestinal physiology. Although the study did not analyze microbiota or bacterial metabolites, the results indicate that

different proteins can modulate the gut-brain axis indirectly through changes in glucose absorption and intestinal cell activation, providing a mechanistic basis for further research on the role of microbiota. Bel Lassen et al. (2021) in a randomized controlled trial (n=107) showed that high-protein supplementation during energy restriction induces visceral fat loss, activates amino acid metabolism by gut microbiota, and demonstrates correlation between microbiota changes and metabolic improvement.

3.6 Effects on Body Composition and Lipid Metabolism

3.6.1 Preferential Fat Mass Loss

High-protein diets promote preferential loss of adipose tissue while preserving muscle mass, which is crucial for maintaining metabolism. In a 6-month RCT in individuals with prediabetes, Rodrigo-Carbó et al. (2024) showed that low-calorie high-protein diets (30%E), regardless of protein source, reduced body weight by an average of 8.1kg, with as much as 92% of weight loss coming from adipose tissue. These diets preserved fat-free mass, reduced waist circumference by 9cm, and decreased visceral fat by 15-20%. Mechanisms of preferential fat mass loss include thermogenesis, as protein has the highest thermic effect (20-30% vs. 5-10% for carbohydrates, 0-3% for fats). Another mechanism is muscle mass protection, as higher protein intake during caloric deficit minimizes muscle catabolism. Additionally, increased fat oxidation occurs, as high glucagon concentration promotes lipolysis and fatty acid oxidation. Zhang et al. (2022) showed that a high-protein diet in individuals with type 2 diabetes reduced hepatic fat - in the entire cohort, intrahepatic lipid content (IHL) decreased by an average of ~42% (from 15.4% to 8.8%), and in the subgroup with the greatest improvement - by as much as ~65%.

3.6.2 Adipokine Modulation

Elahikhah et al. (2024) showed that MPC supplementation in women on a weight-loss diet increased adiponectin levels and decreased leptin levels, which combined with adiponectin increase indicates improved adipokine profile. Moreover, it improved metabolic profile, including insulin resistance and selected lipid parameters (LDL decrease, HDL increase).

3.7. Effects in Specific Clinical Populations

3.7.1 Type 2 Diabetes

Sridonpai et al. (2021) in a crossover RCT compared a multi-component high-protein beverage with a normal breakfast in individuals with type 2 diabetes. The high-protein beverage reduced postprandial glycemia by 35%, increased active GLP-1 by 87%, reduced insulin by 22% (which despite better glycemic control suggests improved sensitivity), and increased satiety. Skytte et al. (2021) investigated carbohydrate restriction (<30g/day) in patients with type 2 diabetes. After 6 weeks, a 53% reduction in postprandial insulin response, 44% reduction in postprandial glycemia, improved beta cell function (increased disposition index), increased post-meal GLP-1 and GIP secretion, and improved satiety despite restriction of the main macronutrient (carbohydrates) were observed.

3.7.2 Heart Failure with Diabetes

Evangelista et al. (2021) conducted the Pro-HEART study in patients with heart failure, diabetes, and overweight. A high-protein diet (30%E) was compared with a standard diet (15%E) for 12 weeks. Unexpectedly, both diets were equally effective in weight reduction (3.6kg in the HP group vs 2.9kg in the SP group), and the difference between groups was not statistically significant. However, the high-protein diet caused significantly greater reduction in glycated hemoglobin (HbA1c), cholesterol, triglycerides, and systolic and diastolic blood pressure compared to the standard diet. Conclusion: The results suggest that a high-protein diet may be more effective in reducing cardiometabolic risk in this population.

3.7.3 Post-Bariatric Surgery Individuals

Although none of the analyzed articles directly concerned the post-bariatric population, some studies using meal replacement diets (TDR/MR) provide potential clinical conclusions. Oliveira et al. (2022) showed that short-term (32h) high-protein total meal replacement diet was well tolerated and modulated postprandial GLP-1 and PYY response, which may have implications in preoperative preparation or in the postoperative period, where satiety signals are significant.

3.7.4 Sex Differences

Wang et al. (2022) in a mouse study investigated the effects of diets with different protein content (12%, 21%, 60%E) on lipid metabolism and gut microbiota depending on sex. Key findings were as follows: in males, the 60% protein diet reduced body weight, visceral fat, and improved lipid profile most effectively; in females, however, effects were less pronounced, with smaller body weight reduction. Sex differences in microbiota

response to protein were also observed, where males showed greater changes in protein-fermenting bacteria, as well as differences in leptin and adiponectin hormone responses between sexes. Mechanisms of potential sex differences include the fact that sex hormones (estrogens, testosterone) modulate protein metabolism, and there are differences in body composition (men have more muscle mass), differences in expression of amino acid-metabolizing enzymes, and differences in gut microbiota.

3.8. Protein Dose and Timing Optimization

3.8.1 Dose

Lesgards (2023) in a review indicates the existence of a dose-response effect for whey protein consumed before a meal (preload). The author cites results illustrating this relationship in reducing postprandial glycemia: a 15g dose reduces postprandial glycemia by approximately 13%, while a 27.6g dose reduces glycemia by approximately 21%, indicating intensification of the effect with increasing dose. Regarding dosing and timing, doses used in studies covered a wide range of whey protein, including effective doses of 27.6g and 55g. Optimal effects were achieved when whey protein (e.g., 55g dose) was consumed 15 minutes or 90 minutes before a meal. Generally, studies used 25-35% energy from protein in the total diet or 1.2-2.0g/kg/day. Supplements used 20-70g protein per serving, while in the preload protocol (protein before meal) 10-55g protein.

3.8.2 Timing

Oliveira et al. (2021) investigated the timing of a high-protein shake relative to exercise: consumption of the shake (25g protein) after exercise increased fat oxidation in the post-training period (greater total fat oxidation vs. control), increased postprandial GLP-1, and decreased post-exercise hunger increase. Ultimately, it improved postprandial metabolic profile through greater incretin response, which may support appetite regulation. Subgroup analysis in the meta-analysis on whey protein preload showed that the timing of its consumption is crucial for optimizing metabolic effects (Smedegaard et al., 2023). When consumed 5-10 minutes before a meal, moderate glycemia-lowering effects were observed, which were statistically less pronounced compared to longer intervals (Smedegaard et al., 2023). The time of 15-30 minutes (up to 40 minutes) before a meal was recognized as optimal (Smedegaard et al., 2023), as researchers noted the greatest reduction in postprandial glycemia (measured as iAUC, i.e., area under the curve) at that time (Smedegaard et al., 2023), with the key mechanism being maximal stimulation of incretin hormones such as GLP-1 (Smedegaard et al., 2023). At intervals exceeding 30 minutes (above 40 minutes) before a meal, hypoglycemic effects may be maintained; however, the meta-analysis authors indicate that using such a long time interval may be less practical in daily use (Smedegaard et al., 2023). The clinical implication is that consuming protein 15-30 minutes before the main meal (particularly breakfast or lunch) may be an effective strategy for individuals with type 2 diabetes or obesity.

3.9. Amino Acids and Molecular Mechanisms

3.9.1 Specific Amino Acids

Yanagisawa (2022) in a review explained how individual amino acids affect insulin and glucagon secretion. Insulinotropic amino acids include leucine, which is the strongest stimulator of insulin secretion and activates mTOR in pancreatic beta cells; isoleucine and valine, which synergistically with leucine stimulate insulin secretion; arginine, stimulating insulin secretion in a glucose-dependent manner; and lysine and alanine, exhibiting moderate insulinotropic effects. Among glucagonotropic amino acids, arginine stands out as the strongest stimulator of glucagon secretion from pancreatic alpha cells; alanine, possessing a strong glucagonotropic effect and being a substrate for gluconeogenesis; and glutamine, which stimulates both glucagon and GLP-1 secretion. The molecular mechanism of action consists of amino acids being transported into pancreatic cells through specific transporters (LAT1, SNAT2), increasing the ATP/ADP ratio, which leads to membrane depolarization and calcium entry. Moreover, amino acids activate signaling pathways (mTOR, AMPK) and modulate expression of genes associated with secretory cell function.

3.9.2 Whey Protein vs. Casein vs. Plant Protein

Whey protein is characterized by very rapid absorption. Compared to other milk proteins, it induces a stronger acute effect on secretion of incretin hormones such as GLP-1, leading to greater insulin stimulation. It is also most effective in reducing postprandial hyperglycemia (Lesgards, 2023). Casein is absorbed more slowly compared to whey because it forms a gel ("clot") in the stomach, leading to slower digestion (Lesgards, 2023). It causes less pronounced GLP-1 and GIP hormone response and promotes longer-lasting satiety (Lesgards, 2023). It may be better for nocturnal muscle protein synthesis. Plant protein is characterized by

variable amino acid profiles (often lower in leucine). Neacsu et al. (2021) showed that hemp induces the highest GLP-1 response, while buckwheat most strongly increases satiety, although other plant sources were neither as strong as hemp (GLP-1) nor as buckwheat (satiety). These sources, however, may provide additional benefits from fiber and phytochemicals. Jiang et al. (2022) compared high-protein diets based on pork vs. soy protein in obese mice. Both diets were effective, but soy protein showed better effects on gut microbiota and greater visceral fat reduction, while pork protein led to better muscle mass retention. Importantly, both sources modulated appetite hormones and improved metabolic profile.

3.10. Interactions with Other Dietary Components

3.10.1 Protein + Carbohydrates (Glycemic Index)

The DIOGENES study (Zhu et al., 2021) compared four diets in a 6-month weight maintenance phase. Among the results, it was found that the combination of high protein + low GI was most effective in hunger suppression in the weight maintenance phase; however, it was not more effective in maintaining weight loss compared to the three other diets. It was found that effects were additive, meaning that each factor (protein, GI) independently contributed to success.

3.10.2 Protein + Fiber

Ni et al. (2022) in a mouse study with type 2 diabetes compared four diet groups. Results indicated that the protein + fiber combination had the strongest effect, leading to the greatest improvement in glycemic control, greatest gut microbiota modulation, increased SCFA production, and highest GLP-1 levels. The mechanism of this action is based on the fact that fiber fermented by microbiota generates SCFAs, which directly stimulate L cells to secrete GLP-1 (through FFAR2/3 receptors), improve gut barrier integrity, and have anti-inflammatory effects; simultaneously, protein provides amino acids stimulating GLP-1 and also changes microbiota composition toward fiber-fermenting bacteria.

3.10.3 Protein + Calcium

Watkins et al. (2023) emphasized the synergistic role of calcium in protein-mediated GLP-1 secretion: calcium from dairy products activates the calcium-sensing receptor (CaSR) on L cells. CaSR synergistically cooperates with amino acid receptors in GLP-1 stimulation. Dairy products (particularly whey) may be more effective than isolated protein due to calcium content.

3.11. Safety and Potential Concerns

3.11.1 Kidney Function

Mensink (2024) indicates that in individuals with normal kidney function, there is no need to modify habitual protein intake, which in Western populations typically amounts to 1.0–1.5g/kg/day and is considered safe. In individuals with chronic kidney disease (CKD), reduced protein intake is recommended - approximately 0.8g/kg body weight - according to current guidelines. None of the analyzed articles reported significant adverse effects associated with high-protein diets in individuals without CKD.

3.11.2 Long-Term Effects

Most studies on high-protein diets in mammals concern short or medium-term periods (up to 12 months). Studies on Drosophila (Yoshinari et al., 2024; Ahrentløv et al., 2025) show that both excessively low and excessively high dietary protein content shorten lifespan, and the optimal nutrient range is narrow. In the fruit fly model, it was shown that very high protein intake is harmful (including through excessive activation of metabolic pathways and hyperammonemia). Consequently, gut hormones (Tk, CCHa1) form a protein satiety system that protects against protein overconsumption, and proper balance between protein and carbohydrates is crucial for longevity. In humans, long-term effects (beyond 5 years) of high-protein diets still require research.

3.11.3 Individual Variability

Kempf et al. (2022) demonstrated significant individual variability in response to diet: approximately 1/3 of participants (lowest tertile) were characterized by smallest leptin reduction and less weight loss. Early biomarkers such as leptin reduction after 1 month allow identification of "responders," suggesting that diet personalization based on early biomarkers can improve treatment efficacy. Factors influencing individual response include leptin resistance status and leptin-insulin relationships (discussed as potential mechanisms of differences between participants). Moreover, metabolic and hormonal factors may also modulate response, although they are not analyzed in detail in this study. In other areas (genetics, microbiota, mitochondria, sex hormones, age), these are possible factors known from the literature but not directly investigated in ACOORH.

4. Discussion

4.1 Impact of High-Protein Diets on Hormonal Axes: Summary of Key Mechanisms

The aim of this systematic review was to analyze the impact of high-protein diets on the profile of gut and metabolic hormones, particularly in the context of appetite regulation, glucose metabolism, and body weight management in the years 2021–2025. The results of studies included in the review consistently indicate a beneficial effect of high-protein diets on a range of hormonal and metabolic parameters. The most significant and consistent effects concerned postprandial glycemic control and satiety regulation. The observed increased secretion of glucagon-like peptide-1 (GLP-1), reaching in various studies from 20% to 156% increase, combined with improved insulin sensitivity, constitutes the central point of mechanisms underlying the pro-health effects of protein. Additionally, a reduction in ghrelin and leptin levels was observed, which further supports the role of high-protein diet in appetite modulation and energy control.

4.2 GLP-1 as a Central Hormone in the Mechanisms of Action of High-Protein Diets

Gut hormones, and especially GLP-1, play a key role in the regulation of food intake and glucose metabolism. The action of GLP-1 consists of influencing insulin secretion, slowing gastric emptying, and enhancing the feeling of satiety. Protein, particularly whey, is a powerful modulator of GLP-1 secretion, acting through multiple mechanisms.

4.2.1 Mechanisms Responsible for Protein-Mediated GLP-1 Secretion:

1. Calcium-dependent mechanism: This mechanism is based on the synergistic action of amino acids and calcium. Protein provides both amino acids, which are a nutritional signal, and calcium, which cooperate in stimulating intestinal L-cells to secrete GLP-1. The synergistic role of calcium was further emphasized: calcium from dairy products activates the calcium-sensing receptor (CaSR) on L-cells. CaSR activation synergistically cooperates with amino acid receptors, which significantly intensifies GLP-1 stimulation. Consequently, dairy products, and especially whey, may be more effective in stimulating GLP-1 than isolated protein, which is attributed precisely to the presence of calcium. This finding has fundamental significance for optimizing dietary composition in metabolic therapies.
2. Receptor mechanism: GLP-1 secretion is strongly dependent on the activation of receptors on the surface of enteroendocrine cells. Amino acids act through activation of taste receptors (T1R1/T1R3), which are sensitive to umami, and the aforementioned CaSR receptor. This dual sensory activation ensures a robust and rapid hormonal response to protein consumption.
3. Peptide transport: A complementary mechanism is the direct transport of protein digestion products. The PepT1 transporter transports di- and tripeptides across the cell membrane of enteroendocrine cells. Inside the cell, transport of these peptides initiates a signaling cascade, which leads to GLP-1 release.

All these molecular pathways – receptor, calcium-dependent, and transport – emphasize why whey proteins have particularly strong action stimulating GLP-1 secretion. Their rapid digestion, delivery of amino acids with high activity (e.g., leucine), and the often accompanying calcium content make them a prototypical nutraceutical in the context of glycemic control and satiety.

4.3 Amino Acids as a Direct Modulator of Insulin and Glucagon Secretion

In addition to indirect effects through gut hormones, amino acids derived from protein digestion exert a direct and strong influence on pancreatic alpha and beta cells. This influence is highly specific to individual amino acids, which has critical significance for understanding their role in maintaining glucose homeostasis.

4.3.1 Insulinotropic Amino Acids:

Leucine: Is recognized as the strongest stimulator of insulin secretion. Its mechanism of action includes key activation of the mTOR (mammalian Target of Rapamycin) pathway in pancreatic beta cells, which links nutrient sensing with secretory processes and cell growth.

Isoleucine and Valine: These branched-chain amino acids (BCAA) exhibit synergistic action with leucine, which is typical for proteins with a complete amino acid profile, such as whey.

Arginine: Stimulates insulin secretion in a manner that is, like leucine, to some extent dependent on glucose concentration.

Lysine and Alanine: Exhibit moderate insulinotropic effects, which complements the spectrum of action of protein hydrolysates.

4.3.2 Glucagonotropic Amino Acids:

Arginine: Although it is insulinotropic, arginine is also the strongest stimulator of glucagon secretion from pancreatic alpha cells. This dichotomous role of arginine is likely of key significance for preventing

postprandial hypoglycemia, which is an important element of the action of high-protein diets, particularly in the context of improved insulin sensitivity.

Alanine: Exhibits a strong glucagonotropic effect. Its metabolic significance is dual, because it also serves as an important substrate for gluconeogenesis, helping to stabilize blood glucose levels in the period between meals.

Glutamine: In addition to stimulating glucagon secretion, glutamine also stimulates GLP-1 release. This ability to simultaneously modulate the gut-pancreatic axis makes it an important amino acid in the metabolic context.

4.4 Molecular Mechanism:

The secretion of pancreatic hormones in response to protein is a highly organized molecular process. Amino acids are transported to pancreatic alpha and beta cells through specific transporters, such as LAT1 and SNAT2. Intracellularly, amino acid metabolism leads to an increase in the ATP/ADP ratio, which is a key energy signal. The increase in this ratio causes closure of ATP-dependent potassium channels, leading to cell membrane depolarization and consequently to calcium entry. The entry of calcium ions initiates fusion of vesicles with hormones and their exocytosis (secretion). Moreover, amino acids and their metabolites activate signaling pathways, such as mTOR (particularly by leucine) and AMPK, which are central regulators of cellular metabolism. Long-term, amino acids modulate the expression of genes associated with the function and survival of secretory cells.

4.5 Clinical Implications in Appetite and Body Weight Management

The potential of high-protein diets in controlling body weight is closely related to their impact on satiety and hunger hormones. One of the most influential clinical studies analyzing this relationship is the DIOGENES project, whose results were published by Zhu et al. (2021). This study, conducted on a large cohort (n=768) in 34 European centers, focused on the weight maintenance phase after initial weight loss.

The analysis compared four key dietary strategies in a 6-month weight maintenance phase after weight loss:

High protein/low GI High protein/high GI Low protein/low GI Low protein/high GI

The main and indisputable conclusion was that a diet high in protein combined with a low glycemic index (HP/LGI) was most effective in suppressing hunger in the weight maintenance phase. A significant reduction in perceived hunger (-12% compared to baseline, $p<0.001$) was observed in the HP/LGI group. Also key was the finding that the effects were additive: each factor – both higher protein content and lower glycemic index – independently contributed to greater feeling of satiety and appetite control. This indicates the strength of synergy between these two dietary modifications.

4.6 Satiety and Mechanisms Independent of Basal Ghrelin

An important, though surprising, finding was that despite strong hunger suppression in the HP/LGI group, no significant differences in fasting ghrelin levels were observed between the compared diets. Ghrelin, as the main orexigenic hormone, typically increases after weight loss, signaling hunger and promoting weight regain. The lack of basal ghrelin suppression in the HP/LGI diet suggests that the satietogenic effects of protein are mediated through mechanisms other than regulation of basal levels of this hormone. This is a strong argument for the hypothesis that postprandial, rather than basal, secretion of hormones such as GLP-1, peptide YY (PYY), and cholecystokinin (CCK) (indirectly implicated through GLP-1 mechanisms) is key to protein-induced appetite control. The effects are therefore most apparent in the postprandial context.

4.7 Sex Context and Microbiota: Perspectives from Preclinical Studies

The study by Wang et al. (2022) on a mouse model provides key information regarding sex differences in metabolic response to high-protein diet. In this study on mice, the effects of diets with different protein content (12%, 21%, 60% E) on lipid metabolism and gut microbiota were analyzed. The results showed clear sex dimorphism in response to dietary intervention.

4.7.1 Sex Differences in Metabolic Response:

Males: High-protein diet (60% E) was most effective in reducing body weight, visceral fat, and improving lipid profile.

Females: In this group, these effects were significantly less pronounced, with smaller reduction in body weight and smaller improvement in lipid parameters.

Adipocytokine Hormones: Differences were also observed in the response of leptin and adiponectin between sexes. The altered response of these adipocytokines to high-protein diet may constitute one of the mechanisms underlying the differentiated effectiveness of intervention depending on sex.

4.7.2 Gut Microbiota as a Modulating Factor:

A key mechanistic element identified by Wang et al. (2022) was sex differences in gut microbiota response to increased protein intake. Males showed greater changes in protein-fermenting bacteria. Fermentation of undigested protein in the large intestine leads to production of short-chain fatty acids (SCFA), such as butyrate and acetate, which in turn affect host energetics and gut hormone secretion (e.g., GLP-1). Differences in composition and activity of gut microbiota are therefore a potential mechanism that should be taken into account when analyzing sex dimorphism in metabolic response.

4.7.3 Mechanisms of Potential Sex Differences:

Researchers indicated several probable mechanisms explaining this dimorphism:

Sex hormones: Estrogens and testosterone can directly modulate protein metabolism.

Differences in body composition: Men/males typically have more muscle mass, which can lead to differences in amino acid metabolism.

Differences in enzyme expression: There are differences in the expression of amino acid metabolizing enzymes, which affects the rate of their utilization and signaling.

Differences in gut microbiota: As mentioned, differences in microbiota composition are themselves a modulating factor.

Although this study was conducted on mice, it provides critical context that should be taken into account in future clinical studies on humans, especially in the design of personalized dietary interventions depending on sex. It suggests that the effectiveness of high-protein diets in reducing body weight and visceral fat may be less efficient in women, which may result from subtle interactions between sex hormones, body composition, and gut microbiota.

4.8 Clinical Implications: Protein "Preload" Strategy

In the context of improving glycemic control and insulin sensitivity, a key clinical strategy is protein "preload." This strategy consists of consuming protein before a meal containing carbohydrates, in order to optimize postprandial hormonal response. Studies have shown the effectiveness of doses in the range of 10–55 grams of protein consumed before the main meal. Such an approach utilizes both indirect mechanisms of protein action (GLP-1) and direct ones (insulinotropic amino acids).

In the postprandial context, protein preload is designed to maximize increased GLP-1 secretion. GLP-1, secreted from L-cells, acts on pancreatic beta cells, enhancing glucose-dependent insulin secretion, which is a key mechanism for better glycemic control. Simultaneously, preload provides key insulinotropic amino acids (e.g., leucine, isoleucine, valine). Leucine, through activation of the mTOR pathway, acts as a strong nutritional signal, enhancing insulin response, which is particularly valuable for individuals with insulin resistance and type 2 diabetes.

In light of the additive effects discovered by Zhu et al. (2021), combining protein preload with a carbohydrate source with a low glycemic index constitutes the most comprehensive and effective dietary strategy. Such a combination targets both maximization of incretin secretion and balancing the rate of glucose delivery, which translates to better glycemic control. This in turn supports long-term body weight management and reduces the risk of metabolic complications. Strategic use of whey proteins in preload, due to their strong insulinotropic action and GLP-1 secretion stimulation, appears as a promising therapeutic tool, especially considering the synergistic action of calcium present in dairy products.

4.9 Dual Role of Amino Acids and Future Research Directions

The role of amino acids in modulating the hormonal axis of the pancreas is more complex than just stimulation of insulin secretion. The action of arginine as the strongest stimulator of glucagon from pancreatic alpha cells, parallel to its insulinotropic action, reveals a mechanism of glycemic self-regulation in response to a protein meal. This postprandial increase in glucagon, often observed after protein meals, balances the strong insulin response. This is important for protection against hypoglycemia, which is particularly important in the context of advanced insulin resistance or diabetes, where stimulation of insulin alone could lead to undesirable drops in glucose levels. Moreover, the amino acid alanine, possessing a strong glucagonotropic effect, also serves as a key substrate for gluconeogenesis. This dual function of alanine, supported by glucagon, ensures maintenance of constant glucose supply in the postprandial period and between meals, which is a

mechanism stabilizing glucose homeostasis. Such integration of insulin and glucagon signaling by specific amino acids is evidence that proteins are dynamic modifiers of metabolism, far exceeding simple provision of building material.

4.10 Challenges in Interpreting Satiety Effects

One of the most intriguing findings of the review concerns the discrepancy between subjective feeling of satiety and objective measurements of basal orexigenic hormones. The success of the high-protein/low-GI diet in hunger suppression (-12 vs. baseline) with simultaneous lack of significant changes in fasting ghrelin level prompts reflection on whether satietogenic effects are fully explained by hormones measured at rest. This suggests that the mechanisms of protein action are shifted toward postprandial modulation and central integration of signals. Increased postprandial GLP-1 secretion, which is key to gastric emptying and feeling of satiety, likely plays a dominant role. Moreover, peptide YY (PYY) and CCK, although not described in detail in all fragments, are logically implicated as mediators of postprandial satiety alongside GLP-1. Therefore, greater attention should be paid to the kinetics of postprandial hormone secretion and their interaction with brain centers responsible for appetite control, because these appear key in explaining the observed hunger suppression.

4.11 Role of Sex Differences in Research in Relation to Humans

Preclinical studies by Wang et al. (2022) on mice revealed that the effectiveness of high-protein diets in reducing body weight and visceral fat is sexually dimorphic. Males showed significantly better results in body weight reduction and lipid profile improvement compared to females, in whom these effects were less pronounced. This sex dimorphism is probably deeply rooted in physiological mechanisms.

4.11.1 Possible Mechanisms of Sex Differences:

Modulation by sex hormones: Estrogens and testosterone play modulating roles in protein and lipid metabolism. The levels of these hormones affect fat storage, its distribution, and tissue sensitivity to insulin. In females, lipid regulation may be more resistant to dietary changes than in males.

Differences in body composition and muscle mass: Males, typically possessing greater muscle mass, have a larger pool of metabolically active tissues for amino acid utilization. Greater amino acid utilization can result in more pronounced changes in metabolism, translating to more effective visceral fat loss.

Gut microbiota: The greater changes in protein-fermenting bacteria observed in males suggest that dimorphism in nutrient processing by microbiota may be key. Microbiota metabolism products (e.g., SCFA) have significant impact on energy and hormonal economy. This difference is reinforced by the observation of differences in leptin and adiponectin response between sexes, which further complicates the picture of hormonal response to high-protein diet.

These findings from preclinical studies constitute a strong call for inclusion of sex-dependent analysis in future clinical studies on humans, in order to develop personalized nutritional recommendations maximizing metabolic benefits of high-protein diet. It is necessary to examine to what extent these mechanisms translate to the human population, and also whether modification of protein composition (e.g., whey type) can eliminate these differences.

4.12 Potential Pitfalls and Need for Integration

Although the review indicates broad benefits of high-protein diets in hormonal modulation, it is necessary to exercise caution in formulating general recommendations. Although hunger suppression effects were additive – that is, both high protein and low GI independently contributed to success – it should be remembered about the corrected conclusion from the DIOGENES study: the HP/LGI diet was most effective in hunger suppression, but not in maintaining weight loss compared to other diets in the long term. This distinction is fundamental: although protein intervention can significantly improve short-term appetite control and glycemia, which is necessary for weight loss, its long-term impact on preventing weight regain may be dependent on additional factors, such as diet adherence, physical activity, and individual metabolic differences.

In future studies, beyond focusing on GLP-1 alone, measurements of other key incretins and adipocytokines should be integrated. In light of leucine's action through mTOR, studying biomarkers of this signaling pathway may also be promising, which would allow for more precise monitoring of metabolic response to protein intervention. Moreover, the wide range of dosing in protein preload (10–55 g) suggests the need for further dose optimization based on individual parameters, such as body weight, degree of insulin resistance, or the type of protein used (e.g., whey vs. casein). Finally, there should be pursuit of more long-

term studies that not only analyze hormones at a single time point, but also monitor the durability of observed hormonal changes in the context of long-term metabolic control and body weight.

5. Conclusions

This systematic review of literature from 2021–2025 confirms that high-protein diet is a powerful and multifaceted modulator of the gut-pancreatic and metabolic axis. The identified mechanisms and clinical study results provide solid evidence that protein, particularly whey, is a key nutraceutical in strategies aimed at improving glycemic control and appetite management.

5.1 Key Findings and Metabolic Implications

High-protein diets consistently show a beneficial hormonal profile, characterized by significantly increased GLP-1 secretion (increase of 20–156% in studies) and improved insulin sensitivity.

5.1.1 Incretin and Amino Acid Mechanisms

GLP-1 (Glucagon-like peptide-1) secretion: Protein, particularly whey fractions, stimulates GLP-1 secretion through three key, mutually complementary mechanisms:

Receptor mechanism: Amino acids activate taste receptors (T1R1/T1R3) and calcium-sensing receptor (CaSR) on enteroendocrine cells.

Calcium-dependent mechanism: Calcium from dairy products acts synergistically with amino acids, activating CaSR and potentiating GLP-1 secretion. This makes dairy products, such as whey, potentially more effective than isolated protein.

Peptide transport: The PepT1 transporter enables direct transport of di- and tripeptides into cells, initiating the GLP-1 signaling cascade.

Modulation of pancreatic hormones by amino acids: Amino acids act directly on the pancreas, which regulates glucose homeostasis:

Insulinotropic amino acids: Leucine is the strongest insulin stimulator, acting through activation of the mTOR pathway in beta cells. Leucine, isoleucine, and valine act synergistically.

Glucagonotropic amino acids: Arginine is the strongest stimulator of glucagon from alpha cells, and alanine acts strongly glucagonotropically and is a substrate for gluconeogenesis. This dichotomous regulation is key for glycemic stabilization.

5.2 Clinical Context: Appetite and Weight Maintenance

In appetite management, it proved key that the combination of high protein with low glycemic index was most effective in hunger suppression in the weight maintenance phase after weight loss. The perceived satietogenic effects were additive, meaning that both protein and low GI independently contributed to success in hunger control. An important conclusion is the fact that satietogenic effects of protein are mediated through mechanisms other than basal ghrelin suppression, which emphasizes the significance of postprandial hormonal signaling (GLP-1).

5.3 Sex Context and Future Challenges

Preclinical studies indicate sex differences in response to high-protein diet, with less pronounced effects of body weight and visceral fat reduction in females than in males. These differences are linked to modulation by sex hormones (estrogens, testosterone), differences in body composition (more muscle mass in males), expression of amino acid metabolizing enzymes, and differences in gut microbiota (greater changes in protein-fermenting bacteria in males). This dimorphic response has key significance for future, personalized nutritional counseling.

5.4 Final Conclusions

High-protein diet is a promising strategy in the fight against obesity and type 2 diabetes, mainly due to its ability for better glycemic control and reduction of ghrelin and leptin levels. The action of protein, especially whey, is complex, based on direct stimulation of pancreatic beta cells by amino acids (e.g., leucine through mTOR) and on a powerful, synergistic incretin mechanism (GLP-1), enhanced by calcium.

5.5 Directions of Future Research Should Focus on:

1. Confirmation of sex dimorphism in metabolic and microbiotic response to high-protein diet in a large human population.
2. Optimization of protein "preload" protocols (10–55 g) to maximize postprandial GLP-1 and insulin secretion.
3. Studying the long-term durability of HP/LGI diet effects on body weight, considering that its main strength lies in hunger suppression, and not necessarily in better weight maintenance in the long term compared to other weight maintenance diets.

In light of available evidence, implementation of high-protein diet, particularly in combination with low glycemic index products, constitutes a clinically justified and effective dietary intervention to improve glycemic control and control perceived hunger. Further mechanistic studies, taking into account the complex interaction of amino acids with hormonal axes and gut microbiota, will certainly contribute to creating more precise and effective nutritional recommendations.

Conflict of Interest: Authors declare no conflict of interest.

REFERENCES

1. Ahrentløv, N., Kubrak, O., Frederiksen, A. S., Lassen, M., Malita, A., Koyama, T., John, A., Imig, C., Richter, E. A., Sigvardsen, C. M., Madsen, P. E. H., Halberg, K. V., Nagy, S., Texada, M. J., & Rewitz, K. (2025). Protein-responsive gut hormone tachykinin directs food choice and impacts lifespan. *Nature Metabolism*. 1. Advance online publication. https://doi.org/10.1038/s42255-025-01267-0
2. Ang, T., Mason, S. A., Dao, G. M., Bruce, C. R., & Kowalski, G. M. (2023). The impact of a single dose of whey protein on glucose flux and metabolite profiles in normoglycemic males: Insights into glucagon and insulin biology. *American Journal of Physiology-Endocrinology and Metabolism*, *325*(6), E688–E699. https://doi.org/10.1152/ajpendo.00182.2023
3. Ataeinosrat, A., Haghighi, M. M., Abednatanzi, H., Soltani, M., Ghanbari-Niaki, A., Nouri-Habashi, A., Amani-Shalamzari, S., Mossayebi, A., Khademosharie, M., Johnson, K. E., VanDusseldorp, T. A., Saeidi, A., & Zouhal, H. (2022). Effects of three different modes of resistance training on appetite hormones in males with obesity. *Frontiers in Physiology*, *13*, Article 827335. https://doi.org/10.3389/fphys.2022.827335
4. Bel Lassen, P., Belda, E., Prifti, E., Dao, M. C., Specque, F., Henegar, C., Rinaldi, L., Wang, X., Kennedy, S. P., Zucker, J.-D., Calame, W., Lamarche, B., Claus, S. P., & Clément, K. (2021). Protein supplementation during an energy-restricted diet induces visceral fat loss and gut microbiota amino acid metabolism activation: A randomized trial. *Scientific Reports*, *11*, Article 15620. https://doi.org/10.1038/s41598-021-94916-9
5. Buso, M. E. C., Seimon, R. V., McClintock, S., Muirhead, R., Atkinson, F. S., Brodie, S., Dodds, J., Zibellini, J., Das, A., Wild-Taylor, A. L., Burk, J., Fogelholm, M., Raben, A., Brand-Miller, J. C., & Sainsbury, A. (2021). Can a higher protein/low glycemic index vs. a conventional diet attenuate changes in appetite-regulating hormones and free-living energy intake during weight loss? *Frontiers in Nutrition*, *8*, Article 656322. https://doi.org/10.3389/fnut.2021.640538
6. Dalgaard, L. B., Kruse, D. Z., Norup, K., Andersen, B. V., & Hansen, M. (2024). A dairy-based, protein-rich breakfast enhances satiety and cognitive concentration before lunch in overweight to obese young females: A randomized controlled crossover study. *Journal of Dairy Science*, *107*(4), 2653–2667. https://doi.org/10.3168/jds.2023-24152
7. Deru, L. S., Davidson, L. E., Chamberlain, C. J., Lance, G. R., Gipson, E. Z., Bikman, B. T., Tucker, L. A., Coleman, J. L., & Bailey, B. W. (2023). The effects of exercise on appetite-regulating hormone concentrations over a 36-h fast in healthy young adults: A randomized crossover study. *Nutrients*, *15*(9), Article 2013. https://doi.org/10.3390/nu15081911
8. Dugardin, C., Fleury, L., Touche, V., Ahdach, F., Lesage, J., Tenenbaum, M., Everaert, N., Briand, O., Lestavel, S., Ravallec, R., & Cudennec, B. (2022). An exploratory study of the role of dietary proteins in the regulation of intestinal glucose absorption and hepatic glucose metabolism. *Frontiers in Nutrition*, *9*, Article 814816. https://doi.org/10.3389/fnut.2021.769773
9. Ekberg, N. R., Catrina, S.-B., & Spégel, P. (2024). A protein-rich meal provides beneficial glycemic and hormonal responses as compared to meals enriched in carbohydrate, fat or fiber, in individuals with or without type-2 diabetes. *Frontiers in Nutrition*, *11*, Article 1395745. https://doi.org/10.3389/fnut.2024.1395745
10. Elahikhah, M., Haidari, F., Khalesi, S., Shahbazian, H., Mohammadshahi, M., & Aghamohammadi, V. (2024). Milk protein concentrate supplementation improved appetite, metabolic parameters, adipocytokines, and body composition in dieting women with obesity: A randomized controlled trial. *BMC Nutrition*, *10*, Article 80. https://doi.org/10.1186/s40795-024-00879-1

11. Evangelista, L. S., Jose, M. M., Sallam, H., Serag, H., Golovko, G., Khanipov, K., Hamilton, M. A., & Fonarow, G. C. (2021). High-protein vs. standard-protein diets in overweight and obese patients with heart failure and diabetes mellitus: Findings of the Pro-HEART trial. *ESC Heart Failure*, *8*(2), 1342–1348. https://doi.org/10.1002/ehf2.13213
12. Hajishizari, S., Imani, H., Mehranfar, S., Yekaninejad, M. S., Mirzababaei, A., Clark, C. C. T., & Mirzaei, K. (2022). The association of appetite and hormones (leptin, ghrelin, and insulin) with resting metabolic rate in overweight/obese women: A case–control study. *BMC Nutrition*, *8*, Article 37. https://doi.org/10.1186/s40795-022-00531-w
13. Hira, T., Sekishita, M., & Hara, H. (2021). Blood sampling from rat ileal mesenteric vein revealed a major role of dietary protein in meal-induced GLP-1 response. *Frontiers in Endocrinology*, *12*, Article 689685. https://doi.org/10.3389/fendo.2021.689685
14. Ignot-Gutiérrez, A., Serena-Romero, G., Martínez, A. J., Guajardo-Flores, D., Alvarado-Olivarez, M., & Cruz-Huerta, E. (2024). Proteins and peptides from food sources with effect on satiety and their role as anti-obesity agents: A narrative review. *Nutrients*, *16*(20), Article 3512. https://doi.org/10.3390/nu16203512
15. Jiang, S., Ji, S., Tang, X., Wang, T., Wang, H., & Meng, X. (2022). A comparison study on the therapeutic effect of high protein diets based on pork protein versus soybean protein on obese mice. *Foods*, *11*(9), Article 1251. https://doi.org/10.3390/foods11091227
16. Kempf, K., Schaller, N., Röhling, M., Banzer, W., Braumann, K. M., Halle, M., McCarthy, D., Predel, H. G., Schenkenberger, I., Tan, S., Toplak, H., Martin, S., Berg, A., & ACOORH Study Group. (2022). Early and strong leptin reduction is predictive for long-term weight loss during high-protein, low-glycaemic meal replacement—A subanalysis of the randomised-controlled ACOORH trial. *Nutrients*, *14*(12), Article 2491. https://doi.org/10.3390/nu14122537
17. Lesgards, J.-F. (2023). Benefits of whey proteins on type 2 diabetes mellitus parameters and prevention of cardiovascular diseases. *Nutrients*, *15*(5), Article 1294. https://doi.org/10.3390/nu15051294
18. Mensink, M. (2024). Dietary protein, amino acids and type 2 diabetes mellitus: A short review. *Frontiers in Nutrition*, *11*, Article 1445981. https://doi.org/10.3389/fnut.2024.1445981
19. Neacsu, M., Duncan, G. J., Cantlay, L., Fyfe, C., Anderson, S., Horgan, G., Johnstone, A. M., Russell, W. R., Vaughan, N. J., Multari, S., Haljas, E., & Scobbie, L. (2022). Hemp and buckwheat are valuable sources of dietary amino acids, beneficially modulating gastrointestinal hormones and promoting satiety in healthy volunteers. *European Journal of Nutrition*, *61*(2), 1057–1072. https://doi.org/10.1007/s00394-021-02711-z
20. Ni, Y., Zheng, A., Hu, Y., Rong, N., Zhang, Q., Long, W., Yang, S., Nan, S., Zhang, L., Zhou, K., Wu, T., & Fu, Z. (2022). Compound dietary fiber and high-grade protein diet improves glycemic control and ameliorates diabetes and its comorbidities through remodeling the gut microbiota in mice. *Frontiers in Nutrition*, *9*, Article 959703. https://doi.org/10.3389/fnut.2022.959703
21. Oliveira, C. L. P., Boulé, N. G., Berg, A., Sharma, A. M., Elliott, S. A., Siervo, M., Ghosh, S., & Prado, C. M. (2021). Consumption of a high-protein meal replacement leads to higher fat oxidation, suppression of hunger, and improved metabolic profile after an exercise session. *Nutrients*, *13*(1), Article 155. https://doi.org/10.3390/nu13010155
22. Oliveira, C. L. P., Boulé, N. G., Elliott, S. A., Sharma, A. M., Siervo, M., Berg, A., Ghosh, S., & Prado, C. M. (2022). A high-protein total diet replacement alters the regulation of food intake and energy homeostasis in healthy, normal-weight adults. *European Journal of Nutrition*, *61*(4), 1849–1861. https://doi.org/10.1007/s00394-021-02747-1
23. Rodrigo-Carbó, C., Madinaveitia-Nisarre, L., Pérez-Calahorra, S., Gracia-Rubio, I., Mateo-Gallego, R., Galindo-Lalana, C., Lamiquiz-Moneo, I., Cebollada, A., & Civeira, F. (2024). Low-calorie, high-protein diets, regardless of protein source, improve glucose metabolism and cardiometabolic profiles in subjects with prediabetes or type 2 diabetes and overweight or obesity. *Diabetes, Obesity and Metabolism*. Advance online publication. https://doi.org/10.1111/dom.16013
24. Schuppelius, B., Ost, A.-C., Schüler, R., Pivovarova-Ramich, O., Hornemann, S., Busjahn, A., Machann, J., Kruse, M., Park, S. Q., Kabisch, S., Csanalosi, M., & Pfeiffer, A. F. H. (2024). Alterations in glucagon levels and the glucagon-to-insulin ratio in response to high dietary fat or protein intake in healthy lean adult twins: A post hoc analysis. *Nutrients*, *16*(22), Article 3869. https://doi.org/10.3390/nu16223905
25. Skytte, M. J., Samkani, A., Astrup, A., Frystyk, J., Rehfeld, J. F., Holst, J. J., Madsbad, S., Burling, K., Fenger, M., Thomsen, M. N., Larsen, T. M., Krarup, T., & Haugaard, S. B. (2021). Effects of carbohydrate restriction on postprandial glucose metabolism, β-cell function, gut hormone secretion, and satiety in patients with type 2 diabetes. *American Journal of Physiology-Endocrinology and Metabolism*, *320*(1), E7–E18. https://doi.org/10.1152/ajpendo.00165.2020
26. Smedegaard, S., Kampmann, U., Ovesen, P. G., Støvring, H., & Rittig, N. (2023). Whey protein premeal lowers postprandial glucose concentrations in adults compared with water—The effect of timing, dose, and metabolic status: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, *118*(2), 391–405. https://doi.org/10.1016/j.ajcnut.2023.05.012

27. Sridonpai, P., Prachansuwan, A., Praengam, K., Tuntipopipat, S., & Kriengsinyos, W. (2021). Postprandial effects of a whey protein-based multi-ingredient nutritional drink compared with a normal breakfast on glucose, insulin, and active GLP-1 response among type 2 diabetic subjects: A crossover randomised controlled trial. *Journal of Nutritional Science*, *10*, Article e49. https://doi.org/10.1017/jns.2021.41
28. Thomas, S., Besecker, B., Choe, Y., & Christofides, E. (2024). Postprandial glycemic response to a high-protein diabetes-specific nutritional shake compared to isocaloric instant oatmeal in people with type 2 diabetes: A randomized, controlled, crossover trial. *Frontiers in Clinical Diabetes and Healthcare*, *5*, Article 1399410. https://doi.org/10.3389/fcdhc.2024.1399410
29. Wang, K., Peng, X., Yang, A., Huang, Y., Tan, Y., Qian, Y., Lv, F., & Si, H. (2022). Effects of diets with different protein levels on lipid metabolism and gut microbes in the host of different genders. *Frontiers in Nutrition*, *9*, Article 940217. https://doi.org/10.3389/fnut.2022.940217
30. Watkins, J. D., Koumanov, F., & Gonzalez, J. T. (2023). Protein- and calcium-mediated GLP-1 secretion: A narrative review. *Advances in Nutrition*, *14*(2), 263–279. https://doi.org/10.1093/advances/nmab078
31. Yanagisawa, Y. (2022). How dietary amino acids and high protein diets influence insulin secretion. *Physiological Reports*, *10*(3), Article e15577. https://doi.org/10.14814/phy2.15577
32. Yoshinari, Y., Koyama, T., John, A., Imig, C., Richter, E. A., Sigvardsen, C. M., Madsen, P. E. H., Halberg, K. V., Nagy, S., Texada, M. J., Tanimoto, H., Kobayashi, T., Matsuyama, M., & Niwa, R. (2024). A high-protein diet-responsive gut hormone regulates behavioral and metabolic optimization in *Drosophila melanogaster*. *Nature Communications*, *15*, Article 10506. https://doi.org/10.1038/s41467-024-55050-y
33. Zhang, J., Pivovarova-Ramich, O., Kabisch, S., Markova, M., Hornemann, S., Sucher, S., Rohn, S., Machann, J., & Pfeiffer, A. F. H. (2022). High protein diets improve liver fat and insulin sensitivity by prandial but not fasting glucagon secretion in type 2 diabetes. *Frontiers in Nutrition*, *9*, Article 808346. https://doi.org/10.3389/fnut.2022.808346
34. Zhu, R., Fogelholm, M., Larsen, T. M., Poppitt, S. D., Silvestre, M. P., Vestentoft, P. S., Jalo, E., Navas-Carretero, S., Huttunen-Lenz, M., Taylor, M. A., Stratton, G., Swindell, N., Kaartinen, N. E., Lam, T., Handjieva-Darlenska, T., Handjiev, S., Schlicht, W., Martinez, J. A., Seimon, R. V., Sainsbury, A., Macdonald, I. A., Westerterp-Plantenga, M. S., Brand-Miller, J., & Raben, A. (2021). A high-protein, low glycemic index diet suppresses hunger but not weight regain after weight loss: Results from a large European multicenter study. *Frontiers in Nutrition*, *8*, Article 700149. https://doi.org/10.3389/fnut.2021.685648