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Genetic and dietary predictors for the postprandial glucose response and possible implications of the postprandial metabolic phenotype on weight management

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‘Nature uses only the longest threads to weave her patterns, so each small piece of her fabric reveals the organization of the entire tapestry.‘

- Richard P. Feynman

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ABSTRACT

Postprandial responses to standardized meals show high inter-individual variation, but there is lacking evidence on which factors determine the variability of postprandial metabolism. This work aims to characterize the postprandial glucose response (PPGR), identify determinants for its inter-individual variation, and discuss its clinical implications for personalized nutrition treatment strategies.

To address this research question, the Lifestyle Intervention (LION) study for weight management has been established. Furthermore, a cross-sectional analysis in the *enable* cohort investigated whether the habitual diet (usual intake of food groups and nutrients) is associated with PPGR (glucose area under the curve (AUC)). Healthy adults of three different age groups were recruited. Clinical and metabolic data were assessed at baseline and during an oral glucose tolerance test (OGTT), and data on eating behavior were collected. Additionally, a systematic review was performed to give an overview of associations between single nucleotide polymorphisms (SNPs) and PPGR.

Data from the LION study (N = 272, 64 % women, mean age 45 ± 11 years, mean baseline body mass index (BMI) 34.55 ± 2.85 kg/m²) described a wide range of variation in individual PPGR trajectories and identified two PPGR clusters in people with obesity. Data from the *enable* cohort (N = 459, 50 % women, mean age 55 ± 21 years, mean BMI 26 ± 5 kg/m²) revealed that the intake of cereals and cereal products, as well as the total carbohydrate intake, are associated with glucose AUC and can be regarded as predictors for PPGR. No dietary parameter equally predicts glucose AUC across all three age groups. None of the further examined dietary parameters were associated with glucose AUC. The systematic review included 46 articles in the narrative synthesis. These articles cover 36 cohorts with healthy participants of different ethnic backgrounds and investigated 53 SNPs in 13 gene loci. Results show either no association or no replicable associations of the examined SNPs with glucose AUC.

In conclusion, findings confirm the inter-individual variation in PPGR and suggest that neither habitual diet nor SNPs alone, are able to predict PPGR. It becomes evident that in future research, the assessment of a combination of parameters and their predicting value for postprandial metabolic response is necessary to implement personalized approaches in the treatment of people with obesity.

ZUSAMMENFASSUNG

Die postprandiale Stoffwechselantwort auf standardisierte Mahlzeitentests weist eine hohe interindividuelle Variabilität auf, es ist jedoch noch unklar, welche Faktoren diese Unterschiede beeinflussen. Ziel dieser Arbeit ist es, postprandialen Glukoseantworten (PPGR) zu charakterisieren, Determinanten für ihre Variabilität zu identifizieren und ihre klinischen Implikationen für die Personalisierten Ernährung zu diskutieren.

Zur Beantwortung dieser Frage wurde die Lebensstilinterventionsstudie (LION-Studie) zum Gewichtsmanagement implementiert. Darüber hinaus wurde eine Querschnittsanalyse in der e*nable*-Kohorte durchgeführt, um Assoziationen zwischen der üblichen Ernährung (übliche Aufnahme von Lebensmittelgruppen und Nährstoffen) und der PPGR (Glukosefläche unter der Kurve (AUC)) zu untersuchen. Rekrutiert wurden gesunde Erwachsene aus drei verschiedenen Altersgruppen. Klinische und metabolische Daten wurden nüchtern und während eines oralen Glukosetoleranztests (OGTT) erhoben. Daten zum Essverhalten wurden erfasst. Eine systematische Übersichtsarbeit wurde zusätzlich durchgeführt, um einen Überblick über Assoziationen zwischen Single Nucleotide Polymorphisms (SNPs) und PPGR zu geben.

Daten aus der LION-Studie (*N* = 272, 64 % Frauen, Durchschnittsalter 45 ± 11 Jahre, mittlerer baseline Body-Mass-Index (BMI) 34.55 ± 2.85 kg/m²) beschreiben eine große Variationsbreite individueller PPGR-Verläufe bei Personen mit Adipositas und zwei PPGR-Cluster wurde identifiziert. Daten aus der *enable*-Kohorte (*N* = 459, 50 % Frauen, Durchschnittsalter 55 ± 21 Jahre, mittlerer BMI 26 ± 5 kg/m²) ergaben, dass der Verzehr von Getreide und -produkten sowie die Gesamtkohlenhydratzufuhr mit der Glukose AUC assoziiert sind und als Prädiktoren für PPGR angesehen werden können. Keiner der untersuchten Ernährungsparameter gilt gleichermaßen als Prädiktor für die PPGR in allen drei Altersgruppen. Von den weiter untersuchten Ernährungsparametern war keiner mit der PPGR assoziiert. Die systematische Übersichtsarbeit bezog 46 Artikel in die narrative Synthese ein. Diese Artikel umfassen 36 Kohorten mit gesunden Personen unterschiedlicher ethnischer Herkunft, die insgesamt 53 SNPs in 13 Genloci untersuchten. Die Ergebnisse zeigen entweder keine Assoziation oder keine replizierbaren Assoziationen der untersuchten SNPs mit der Glukose AUC.

Diese Ergebnisse bestätigen die interindividuelle Variabilität der PPGR und führen zu der Erkenntnis, dass die übliche Ernährung oder SNPs alleine nicht ausreichend die PPGR vorhersagen. Zukünftig ist die Erfassung eines breiten Spektrums an Parametern, sowie die Evaluierung deren Vorhersagekraft für die postprandiale metabolische Antwort notwendig, um personalisierte Ansätze in der Behandlung von Personen mit Adipositas umzusetzen.

ABBREVIATIONS

AHEI	Alternate healthy eating index
AIC	Akaike information criteria
AMY	Salivary α-amylase
App	Smartphone application
AUC	Area under the curve
BMI	Body mass index
BP	Blood pressure
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus Disease 2019
CGM	Continuous glucose monitor
DIETFITS	Diet Intervention Examining the Factors Interacting with Treatment Success
DNA	Deoxyribonucleic acid
E%	Energy percent
EFSA	European Food Safety Authority
FFQ	Food frequency questionnaire
GWAS	Genome-wide association studies
GIP	Gastric inhibitory polypeptide
GIPR	Gastric inhibitory polypeptide receptor
HbA1c	Glycated hemoglobin A1c
HC	Hip circumference
HDL-C	High-density lipoprotein cholesterol
KCNJ11	Potassium inwardly rectifying channel subfamily J member 11
LDL-C	Low-density lipoprotein cholesterol
LION	Lifestyle Intervention

NCD	Non-communicable diseases
NIH	National Institutes of Health
OGTT	Oral glucose tolerance test
OGLTT	Oral glucose and lipid tolerance test
OLTT	Oral lipid tolerance test
PAL	Physical activity level
PERSON	Personalized Glucose Optimization through Nutritional Intervention
PCA	Principal components analysis
POUNDS	Preventing Overweight Using Novel Dietary Strategies
PPARγ	Peroxisome proliferator-activated receptor gamma
PPGR	Postprandial glucose response
PREDICT 1	Personalized Responses to Dietary Composition Trial 1
PREVIEW	Prevention of Diabetes Through Lifestyle Intervention and Population Studies in Europe and Around the World
PRISMA	Preferred Reporting Items for Systematic Review and Meta-analyses
PROSPERO	International Prospective Register for Systematic Reviews
RCT	Randomized controlled trial
RMR	Resting metabolic rate
SCFA	Short-chain fatty acid
SGLT1	Sodium-dependent glucose cotransporter 1
SNP	Single nucleotide polymorphism
TAG	Triacylglycerol
TCF7L2	Transcription factor 7-like 2
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio

1 INTRODUCTION

1.1 HEALTH AND THE POSTPRANDIAL STATE

In 2011 a new concept for the definition of health was presented, describing health as the ability of allostasis (Huber et al. 2011). In other words, health is considered as 'the ability to adapt and to self-manage' when faced with physical, mental, or social perturbations to return to a stable homeostatic state (Huber et al. 2011). For this reason, the general health status is shaped by various exogenous and endogenous factors that can disrupt the genetic, immunologic, physical-chemical, structural, psychological, and physiological homeostasis of the human body.

Focusing on the physiological context, health is increasingly being regarded as the response and adaptation of the metabolism to short-term homeostasis-changing stimuli, such as stress, starvation, or food intake, and is often referred to as metabolic flexibility (Fiamoncini et al. 2018; Kardinaal et al. 2015; Goodpaster and Sparks 2017). On that account, the term metabolic flexibility describes health as a dynamic state, with constant efforts of physiology to adapt to environmental changes (Griffiths et al. 2020; Goodpaster and Sparks 2017).

Metabolism has long been solely defined as an individual's energy expenditure (Secor 2009). Accordingly, research on metabolic flexibility has traditionally focused on heat production after meal ingestion, which is induced by an increase in energy expenditure in the postprandial state (Secor 2009). This process was originally coined as *specific dynamic action* by Max Rubner (Secor 2009). Over the years, multiple pathways involved in the human metabolism, such as energy production, biosynthesis, regulation of oxidative stress, inflammation, immune reaction, or repair of deoxyribonucleic acid (DNA) damage were described (Secor 2009; Goodpaster and Sparks 2017; van Ommen et al. 2014). Today, different aspects of metabolism and metabolic flexibility are topics of research. For instance, the determination of postprandial changes in circulating metabolites and their health implications combines nutritional with medical insights and has gained considerable interest (Lépine et al. 2022).

The postprandial state is the period following meal intake, which is characterized by a transition from a steady fasted state to a dynamic fed state (Lépine et al. 2022). The postprandial state encompasses the ingestion and digestion of food, the absorption of nutrients in the small intestine, their uptake in the bloodstream, nutrient assimilation in the cells, and subsequently their usage, storage, or clearance in different organs (Lépine et al. 2022). The release of various metabolites is up- or downregulated in this process, to ensure

optimal modulation of the nutrient influx by the small intestine (Papakonstantinou et al. 2022), and can be referred to as postprandial metabolic response (Sanders 2016).

The postprandial metabolic response offers insights into the functioning and resilience of the human metabolism since regulatory pathways striving for homeostasis (balancing between anabolism and catabolism) run efficiently under healthy conditions (Lépine et al. 2022). In contrast, chronic allostatic overload results in overcompensation through regulatory and adaptive processes to maintain homeostasis of core physiological functions, reducing metabolic flexibility (Kardinaal et al. 2015; Lépine et al. 2022; Huber et al. 2011). Therefore, the characterization of the postprandial state can be regarded as an approximation to quantify metabolic flexibility and health status (van Ommen et al. 2014).

1.2 THE POSTPRANDIAL METABOLIC RESPONSE AS A TOOL TO MEASURE HEALTH

There are several types of tests applied in science, aiming to challenge physiological homeostasis for the evaluation of an individual's metabolic flexibility. These "challenge tests" make use of different external stimuli as homeostasis modifying factors, triggering different physiological processes involved in homeostasis (Stroeve et al. 2015). Challenge tests mentioned in the literature include physical activity, cold exposure, skin prick tests, fasting, and meal intake, among others (Krug et al. 2012; Zeevi et al. 2015; Vis et al. 2015; van Ommen et al. 2014). The response to such a challenge can be evaluated as a "biomarker" or "proxy" representing health status since several physiological processes are triggered to restore homeostasis (van Ommen et al. 2014). To measure the postprandial state, meal challenges are applied.

The pioneering work of Krug and colleagues described how the human metabolism changes depending on diverse challenge tests (Krug et al. 2012). Results of this work showed how each challenge test provoked an expected metabolic response related to the applied external trigger, further emphasizing the validity of challenge tests to measure metabolic flexibility (Krug et al. 2012). The basis of such challenge tests lies in the assumption that a reduced metabolic flexibility is present, if the observed metabolic response is altered when compared to the expected adequate response (Figure 1) (Vis et al. 2015; Lépine et al. 2022). In that regard, a metabolic response is altered either when its extent or the recovery is different than would be considered appropriate (Figure 1) (Stroeve et al. 2015).

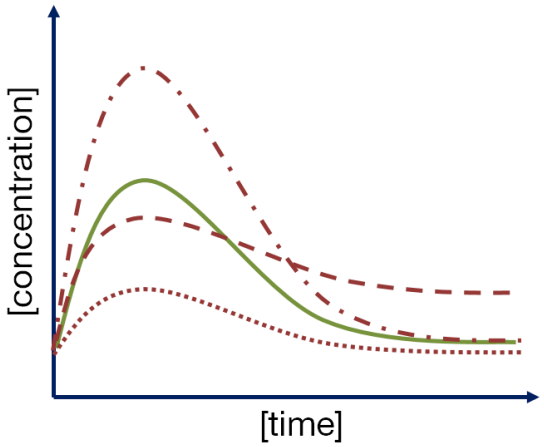

Figure 1 | Schematic overview of metabolic responses to a challenge test.
Responses are shown graphically as concentration changes of the metabolite of interest over time. Red dashed lines represent attenuated responses in comparison to the expected response (green solid line).

1.2.1 MEAL CHALLENGES

The application of meal challenges is well suited to evaluate metabolic flexibility. It is a straightforward and standardized procedure that mimics a nutritional stress response and can be used to observe postprandial metabolic changes and identify individuals with varied postprandial metabolic responses (Lépine et al. 2022; Stroeve et al. 2015). Indeed, several studies have previously shown aspects of metabolic health that are not apparent in the fasting state through the application of meal challenges (Vis et al. 2015; Krug et al. 2012; Berry et al. 2020; Kardinaal et al. 2015; Pellis et al. 2012).

To date, various types of meal challenges have been described, including single-nutrient tests or multi-nutrient tests with a standardized nutrient composition (Lépine et al. 2022). These meal challenges can either be offered in liquid form as a drink or as a meal with a complex food matrix and are usually normo- or hypercaloric (Table 1).

Hitherto, the most investigated meal challenge is the oral glucose tolerance test (OGTT), which specifically triggers glucose metabolism (Vis et al. 2015; Lépine et al. 2022). The OGTT is considered the method of choice for risk assessment or the diagnostic clarification of diabetes mellitus, a heterogeneous disease clinically manifested by hyperglycemia (American Diabetes Association 2020; Erdős et al. 2021). For a standard OGTT, 75 g glucose in solution is orally administered after an overnight fast, and blood samples are collected through a peripheral intravenous catheter at baseline and 2 hours after glucose ingestion (Chen et al. 2018). This test triggers the glycemic response, which encompasses an immediate rise in blood glucose due to its absorption from the small intestine into the bloodstream, followed by the return of blood glucose to fasting levels over time, as a consequence of the transport

of glucose to different organs, stimulating glycolysis, and inhibiting ketogenesis, proteolysis, and lipolysis (Sanders 2016; Lépine et al. 2022). In the clinical setting, the postprandial plasma blood glucose concentration measured 2 hours after the OGTT is used for the classification of glucose tolerance (< 140 mg/dl in normal glucose tolerance; between ≥ 140 and < 200 mg/dl in impaired glucose tolerance, and ≥ 200 mg/dl in diabetes mellitus) (American Diabetes Association 2020). In research, however, blood samples are usually collected at additional time points during the OGTT and postprandial changes of further metabolites might be of interest to characterize the postprandial metabolic response and evaluate metabolic flexibility (Chen et al. 2018).

Table 1 | Examples of meal challenges commonly used in research.

	Single-nutrient	**Multi-nutrients**
Drinks	OGTT (Berry et al. 2020) OLTT (Fatima et al. 2018)) Protein shakes (Berry et al. 2020)	Multi-nutrient drinks (Adamska-Patruno et al. 2019; Artegoitia et al. 2021) High-fat milkshake (Kardinaal et al. 2015) OGLTT (Wopereis et al. 2013) "PhenFlex" test (Wopereis et al. 2017) Chocolate milk (Berry et al. 2020)
Meals	Glucose, fructose (Zeevi et al. 2015) Fiber bars (Berry et al. 2020)	Muffin (Berry et al. 2020) Fast food (Bondia-Pons et al. 2014) White bread with butter (Zeevi et al. 2015) Whole meals with varying nutrient compositions (Berry et al. 2020; Atkinson et al. 2018) Mediterranean or vegan diet (Stroeve et al. 2015)

Abbreviations: OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; OGLTT, oral glucose and lipid tolerance test; PhenFlex, is the acronym for phenotypic flexibility.

The execution of other meal challenges is identical to the above-described OGTT procedure. However, each type of meal challenge can trigger different postprandial metabolic pathways, making a different set of metabolites the target of the investigation. For instance, the oral lipid tolerance test (OLTT) can simulate a fatty acid overload and elicits a postprandial response of triacylglycerol (TAG), cholesterol, or C-reactive protein, reflecting early stages of inflammatory or immune responses (Fatima et al. 2018). Furthermore, the nutrient composition of meal challenges also defines the duration of the postprandial phase (Secor 2009). Meal challenges with a simple composition are highly standardized, and usually of shorter duration, but fail to showcase the complexity of the postprandial metabolism, since it only triggers specific pathways (Stroeve et al. 2015). More complex metabolic responses are

evoked by multi-nutrient challenges, but test duration increases and the metabolic responses are difficult to interpret (Stroeve et al. 2015). Based on these factors, the appropriate meal challenge test needs to be selected accordingly.

This work mainly focuses on the OGTT as an approximation to measure metabolic flexibility, since it is a highly standardized meal challenge, that triggers the well-studied and quick-responding glucose metabolism. Accordingly, in the following sections, the term postprandial metabolic response refers to the response of an unspecified metabolite to an undefined meal challenge, whereas the postprandial glucose response (PPGR) indicates the glucose rise after an OGTT.

1.2.2 Evaluation of the postprandial metabolic response

The amplitude and duration of the postprandial metabolic response to external stimuli are key parameters indicating the extent of an individual's resilience, thus suggesting a person's health status (van Ommen et al. 2014). To get information on the whole postprandial metabolic response, it is crucial to collect samples at different time points during the postprandial state (Vis et al. 2015). For this, blood is the most common sample, but urine or breath samples can also be collected (van Ommen et al. 2014). The duration of the test, as well as the total number of collected samples, vary depending on the nutrient composition of the applied meal challenge, the metabolite of interest, and the research question (Lépine et al. 2022).

The postprandial metabolic response can be analyzed based on the absolute concentration of single or multiple metabolites at one or several time points (Vis et al. 2015). The 2-hour glucose concentration is a prominent example of a single time-point parameter of the PPGR, mostly used to classify glucose tolerance status (Erdős et al. 2021). Similarly, several indices can be calculated based on a single time-point concentration or the average postprandial concentration (Erdős et al. 2021). These indices are used to estimate whole-body or tissue-specific postprandial metabolic response, such as the Matsuda index quantifying insulin resistance (Erdős et al. 2021). Measures such as peak time, or the maximum and minimum postprandial concentrations can also be considered (Vis et al. 2015).

However, single time-point parameters fail to consider the time-dependent dynamics of the postprandial metabolic response (Erdős et al. 2021). For this purpose, further parameters such as the amplitude, slope, or area under the curve (AUC), are used as quantitative measures of the postprandial metabolic response of a metabolite during a set timeframe after intake of a test meal (Erdős et al. 2021; Vis et al. 2015).

Multivariate response analysis can also be performed to assess simultaneous reactions of different components of postprandial metabolism (Vis et al. 2015). In that respect, subgroups can be identified based on the set of metabolites found in the blood during the postprandial state, also named “metabotypes” (Hillesheim and Brennan 2020). Finally, clustering methods are also often applied for the identification of homogenous postprandial trajectories and the characterization of phenotypically similar or different subgroups (Erdős et al. 2021; Vis et al. 2015; Stroeve et al. 2015).

1.3 DETERMINANTS OF THE POSTPRANDIAL GLUCOSE RESPONSE

In the past, studies have observed that postprandial metabolic responses to standardized meals show high inter-individual variability (Berry et al. 2020; Krug et al. 2012; Zeevi et al. 2015), possibly providing crucial information on the metabolic flexibility of different postprandial metabolic phenotypes (Vis et al. 2015). As stated previously, the postprandial metabolic response is determined by the transition from a catabolic to an anabolic state, involving digestive and absorptive mechanisms, such as the production of hormones and enzymes from the gastrointestinal tract and pancreas, the secretion of bile from the liver, the absorption of nutrients in the gut, the uptake of nutrients in the bloodstream, and their delivery to target organs (Fiamoncini et al. 2018). Any factors involved in these pathways, are conceivable determinants of the postprandial metabolic response and might influence health in the long term. Early studies evaluating possible determinants found that diverse factors, including characteristics of the meal (e.g., meal composition, portion size, meal temperature), the individual (e.g., age, sex), and the environment (e.g., ambient temperature) directly affect the duration and amplitude of the postprandial metabolic response (Secor 2009).

The PPGR after an OGTT is an example of a postprandial metabolic response. Factors, such as hormone status (Bermingham et al. 2022; Chia et al. 2018), medication (Christensen et al. 2018a), circadian rhythm (Papakonstantinou et al. 2022), environment (Dumke et al. 2015), psychological factors (Nowotny et al. 2010), diet, physical activity (Slentz et al. 2016), blood pressure (Berry et al. 2020), fasting levels of clinical biomarkers (Berry et al. 2020), the gut microbiome (Asnicar et al. 2021), body composition (Chia et al. 2018), and genotype, have been reported to influence the PPGR (Figure 2). In the following section, genetic and lifestyle determinants are presented in more detail.

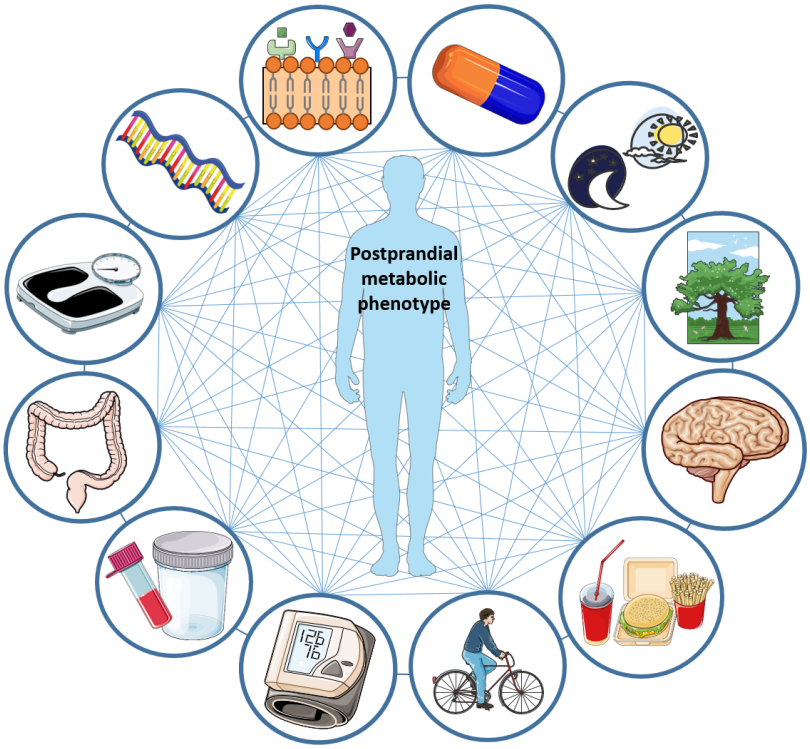

Figure 2 | Determinants affecting postprandial metabolic phenotype.
In a clockwise direction starting from the top, the icons refer to the following determinants: hormone status, medication, circadian rhythm, environment, psychological factors, diet, physical activity, blood pressure, fasting levels of clinical biomarkers, microbiome, body composition, and genotype. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

1.3.1 LIFESTYLE

Diet and physical activity are external determinants of health and disease (Krug et al. 2012). Physical activity, for example, requires a certain degree of metabolic flexibility to match the increased energy expenditure and shift of fuel oxidation (Goodpaster and Sparks 2017). Nonetheless, this work focuses on the diet, since it has been reported that differences in the physical structure and nutrient content of an individual's diet can influence the digestion and absorption of nutrients (Sanders 2016). Indeed, many studies have described that the macronutrient composition of an ingested meal affects the acute postprandial metabolic response (Matone et al. 2015; Meng et al. 2017; Sato et al. 2018; Papakonstantinou et al. 2022).

However, it is not entirely clear how the habitual diet affects PPGR in the long term. The hypothesis is that a long-term inadequate intake of total energy or specific food compounds might disrupt postprandial metabolism, triggering adaptation mechanisms beyond the scope of a "healthy" metabolic flexibility, which can turn chronic and contribute to the development of diseases (van Ommen et al. 2014; Kardinaal et al. 2015; Desmarchelier et al. 2019). There is considerable research interest mainly focusing on the effects of diverse dietary patterns on

postprandial glycemia and insulin resistance, as an intervention for the prevention or treatment of individuals with diabetes mellitus (Papakonstantinou et al. 2022). For example, it has been reported that an increased intake of specific food components, such as dietary fiber, full-fat dairy products, or calcium, can lower insulin resistance (Papakonstantinou et al. 2022).

Research is increasingly interested in assessing distinct subgroups of individuals characterized by similar variations of PPGR. For example, it has been demonstrated that people following a less healthy dietary pattern showed a reduced postprandial blood glucose clearance after a multi-nutrient meal challenge (Fiamoncini et al. 2018). Furthermore, people with an increased intake of red and processed meat, alcoholic beverages, refined grains, and sugar-sweetened beverages were observed to have a decreased glucose tolerance (based on the 2-hour glucose level) after an OGTT (Pestoni et al. 2021; Riedl et al. 2020). However, in PREDICT 1 habitual diet was found to explain 0.06 % of PPGR, whereas meal composition and meal context explained the variance of PPGR to a larger extent (15.4 % and 7.7 %, respectively) than anticipated (Berry et al. 2020). In the same study, increased fat, fiber, and protein intake were described as predictors of a reduced glucose AUC (Berry et al. 2020).

Overall, the physiological processes of individual dietary components involved in postprandial metabolic response are diverse and not yet fully explored (Desmarchelier et al. 2019). On this account, there is still limited knowledge on whether the habitual diet has long-term effects on PPGR or if an improvement of the habitual diet translates into improvements in postprandial metabolism.

1.3.2 GENOTYPE

The underlying assumption on how genetic characteristics can affect the postprandial metabolic response is that variations of genes influencing mechanisms involved in the digestion, absorption, or assimilation of food components might subsequently cause changes in postprandial metabolism. For example, a mutation in the gene coding for the sodium-dependent glucose cotransporter 1 (SGLT1) located in the small intestine can cause the congenital disorder known as glucose-galactose malabsorption (Sanders 2016). Furthermore, genetic defects affecting insulin function, such as its glucose-stimulated secretion, its granule docking, or its post-transcriptional processing, have been suggested to play a role in glucose homeostasis (Grarup et al. 2014).

The most well-known disorder associated with hyperglycemia is diabetes mellitus (American Diabetes Association 2020). Although some subcategories of diabetes mellitus are caused by a variation in a single gene (American Diabetes Association 2020), other subcategories seem

to be associated with several single nucleotide polymorphisms (SNPs). Therefore, most research assessing the influence of the genotype on the PPGR focus on the 2-hour glucose level, because this parameter is considered a clinical measure to assess diabetes risk. A meta-analysis of nine genome-wide association studies (GWAS) including 15,234 participants reported on five gene loci associated with 2-hour glucose levels after an OGTT (Saxena et al. 2010). For example, this publication first reported that a variation in the *gastric inhibitory polypeptide receptor (GIPR)* gene was associated with reduced regulatory function of the gastric inhibitory polypeptide (GIP), an incretin hormone that stimulates insulin response after glucose is orally administered, resulting in an increased 2-hour glucose level (Saxena et al. 2010). The t*ranscription factor 7-like 2 (TCF7L2)* gene, which encodes for a transcription factor in the Wnt signaling pathway, is another genetic contributor to an increased risk for diabetes mellitus (Jin and Liu 2008). Carriers of the risk allele have been reported to be characterized by impaired insulin secretion, reduced incretin effects, and increased hepatic glucose production (Lyssenko et al. 2007). Accordingly, variations of the *TCF7L2* gene were reported to be associated with increased fasting and 2-hour glucose levels (Saxena et al. 2010). Variations of the *peroxisome proliferator-activated receptor gamma (PPARγ)* gene were also discussed to contribute to higher diabetes risk. A meta-analysis including data of 32,000 participants without diabetes across 57 studies, found that variations of the *PPARγ* gene significantly correlated with increased insulin resistance in a subgroup of adults with obesity; however, 2-hour glucose levels were not significantly different between carriers and non-carriers of the risk allele (Tönjes et al. 2006).

Overall, there are not many studies evaluating the genetic influence on PPGR as a whole (calculated as glucose AUC). To mention a few, it has been reported that people with higher copy numbers of the *salivary α-amylase* (*AMY1*) gene might have a greater ability to digest high-starch meals since a 15 - 40 % higher PPGR has been observed in affected individuals (Atkinson et al. 2018). Further, results of the Personalized Responses to Dietary Composition Trial 1 (PREDICT 1) revealed that several SNPs derived from GWAS were significantly ($p < 0.05$) associated with postprandial glucose AUC, and explained 9 % of its variation, implying that the genetic fingerprint plays a minor role as a predictor for postprandial responses (Berry et al. 2020). Overall, there is still a lack of robust evidence on associations between genetic variants and postprandial metabolic response, gene-phenotype interactions, and how the genotype may contribute to the individual variance of PPGR.

1.4 RESEARCH INTEREST IN THE POSTPRANDIAL METABOLIC PHENOTYPE

It was observed that the inter-individual postprandial responses are reproducible for the individual (Berry et al. 2020; Bush et al. 2020), indicating that meal challenges are useful tools to distinguish and characterize postprandial metabolic phenotypes of individuals (Vis et al. 2015). The identification of postprandial metabolic phenotypes presents an opportunity to tailor and optimize diagnosis, prevention, treatment, prognosis, and predictions of disease (Chung et al. 2020). However, the postprandial metabolic response is embedded in a larger system determining health (van Ommen et al. 2014). Therefore, to accurately characterize one's health status, phenotypic characteristics are needed to be integrated with other factors contributing to the variation of postprandial metabolism (Vis et al. 2015; Ordovas et al. 2018; Bush et al. 2020; Biesiekierski et al. 2019). This concept, coined as personalized medicine, is gaining momentum as paradigm shifts from evidence-based medicine, which makes use of population-based practice guidelines ("one size fits all" approaches) for the treatment of common diseases in the last decades (Ziegelstein 2017; van Ommen et al. 2017). Factors influencing health can be intrinsic or extrinsic in nature and are modifiable or non-modifiable.

In this context, modifiable factors such as the diet, represent possible impactful targets for interventions regarding the prevention or treatment of diet-related diseases (Ordovas et al. 2018). As proposed by the American Nutrition Association, the foundation for personalized nutrition lies in the prevention, management, treatment, and prognosis of diet-related diseases, or the optimization of health by adjusting nutritional advice according to an individual's circumstances and needs (Bush et al. 2020). This definition makes clear, that personalized nutrition is a multilayered approach to improving health, which in turn is formed based on intrinsic and extrinsic determinants (Ordovas et al. 2018), that are highly inter-connected, and might even interact with or at least influence each other.

Personalized nutrition is gaining particular interest in the treating non-communicable diseases (NCDs), which contribute to over 50 % of the global burden of disease, since unfavorable dietary habits were found to be the strongest factor leading to a higher risk for NCD mortality and morbidity (Afshin et al. 2019; Benziger et al. 2016). An unfavorable diet is a modifiable risk factor, and there is evidence that dietary improvements reduce mortality associated with NCDs (Afshin et al. 2019). As an example, a key dietary strategy to improve insulin resistance is a diet that is capable of delaying gastric emptying or decreasing the glycemic load to reduce insulin secretion and reduce postprandial glucose fluctuations (Papakonstantinou et al. 2022). In that regard, the assessment of the PPGR after glucose load might be useful to identify how a person responds to a specific meal and if the individual response is associated with an

increased risk for later diet-related diseases. Subsequently, based on the acquired results, a diet can be modulated and optimized to improve the glucose response regarding its amplitude and duration (Figure 3) (Stroeve et al. 2015).

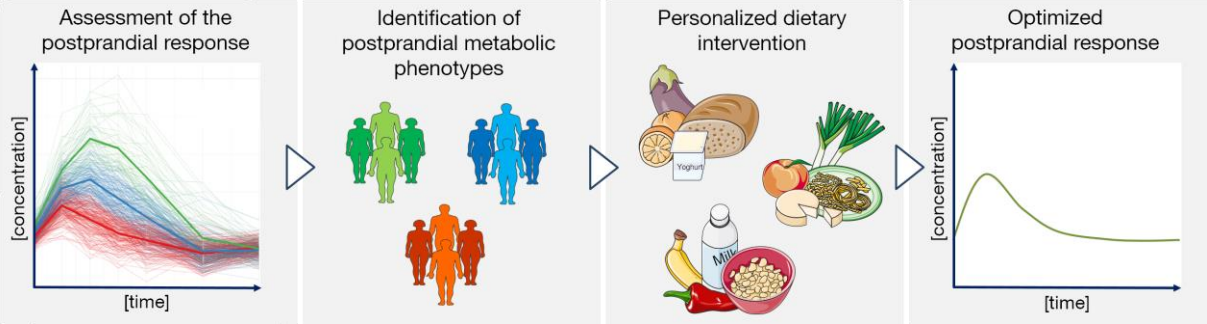

Figure 3 | Overview of the application of personalized nutrition.
Large inter-individual variation can be seen after the postprandial responses of individuals are assessed. The identification of subgroups, characterized by similar or different postprandial responses, may increase the efficacy of dietary interventions. The aim is to modulate diet to these subgroups, to achieve an optimized postprandial response. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

To narrow down the complexity of "personalized nutrition", the focal point of the following sections is to present current research on the characterization of the PPGR and its clinical implications for personalized nutrition strategies to manage obesity.

1.4.1 Personalized nutrition based on the postprandial glucose response

Researchers at the Weizmann Institute of Science have been pioneering research in the area of personalized nutrition based on the characterization of an individual's postprandial metabolic phenotype. Since lifestyle interventions for the treatment of type II diabetes mellitus often fail in the long term, this research group has set itself the mission to evaluate personalized treatment approaches to optimize glycemic control (Zeevi et al. 2015). Back in 2015, Zeevi *et al.* published an article describing a first-of-its-kind comprehensive study on personalized nutrition. In total, 800 healthy adults aged 18 - 70 years were included and deep phenotyping (based on the gut microbiome, blood tests, questionnaires, anthropometrics, and food diaries) was performed (Zeevi et al. 2015). Further, standardized meals were provided for each participant and PPGR was measured utilizing a continuous glucose monitor (CGM), which resulted in high inter-individual PPGR (Zeevi et al. 2015). With the acquired data, computational analysis allowed the identification of determinants of the PPGR (Zeevi et al. 2015). Subsequently, an algorithm was developed, allowing the prediction of an individual's PPGR based on the integration of sociodemographic and microbiological data (Zeevi et al. 2015). The long-term aim was to design personalized diets able to lower an individual's glycemic response. The effectiveness of such a personalized diet was further tested in comparison to a Mediterranean diet concerning their ability to improve postprandial

glycemic control (Ben-Yacov et al. 2021). The personalized diet proved to reduce the time of daily glucose levels over a threshold of 140 mg/dl and glycated hemoglobin A1c (HbA1c) levels by -1.3 ± 1.5 hours/day and -0.16 ± 0.24 %, respectively, which was maintained after 12 months of follow-up (Ben-Yacov et al. 2021). These findings highlight the potential clinical implications of personalized dietary recommendations in the treatment of people with type II diabetes mellitus or as a preventive strategy for individuals at increased risk for the disease.

The more recent PREDICT 1 explores similar relationships, extending the focus on postprandial biomarkers associated with cardiometabolic diseases. This study aimed to comprehensively assess postprandial glucose, insulin, and TAG concentrations and their influencing factors, as well as to predict postprandial metabolic responses based on machine-learning algorithms (Berry et al. 2020). In total, 1,002 twins and unrelated healthy adults aged 18 - 65 years were recruited and were phenotyped by evaluating the postprandial metabolic response to meal challenges (Berry et al. 2020). The variation of the postprandial glucose, insulin, and TAG responses was influenced differently by the evaluated determinants (Berry et al. 2020). Some modifiable factors (e.g., meal timing, exercise, or sleep) were found to play a role as a determinant of postprandial metabolic responses, further highlighting the potential of personalized treatments by adjusting such environmental exposures to an individual's characteristics (Berry et al. 2020). Based on these observations, a machine-learning model was fitted that allowed predictions for postprandial glucose and TAG response (Berry et al. 2020).

The Personalized Glucose Optimization through Nutritional Intervention (PERSON) study is a randomized controlled trial (RCT) that makes use of the OGTT to characterize different phenotypes based on tissue-specific insulin resistance of people living with overweight and obesity (Gijbels et al. 2021). This trial aims to evaluate how the macronutrient composition of an isocaloric dietary intervention affects glucose metabolism and other cardiometabolic risk factors (Gijbels et al. 2021). The results of this study are still pending and are not yet published.

The number of publications focused on the personalization of dietary intervention to improve glucose metabolism highlights the increasing trend towards personalized nutrition and its potential as an effective and comprehensive strategy to improve the health of people at risk, in comparison to established "one size fits all" dietary strategies. It is evident that there are many trials targeting other diet-related diseases and their associated metabolic consequences.

1.4.2 PERSONALIZED NUTRITION AS A STRATEGY FOR OBESITY MANAGEMENT

A recent report by the World Health Organization (WHO) concluded that almost 60 % of the European population is affected by either overweight or obesity, a complex chronic disease of multifactorial etiology that can have significant health consequences (World Health Organization 2022). Lifestyle-based interventions for weight loss and maintenance are widely established in guidelines for the prevention and treatment of overweight and obesity. However, weight loss and maintenance success are highly individual, indicating that strategies of weight management based on a “one size fits all” approach are not effective (Astrup and Hjorth 2018) and have failed, so far, to reduce the ever-increasing prevalence of overweight and obesity. Obesity has been described to be caused by an imbalance of energy intake and energy expenditure, resulting in an excessive accumulation of adipose tissue. To date, the body mass index (BMI) is considered a simple, fast, and inexpensive way to identify obesity, although this method is recognized to be flawed (Frühbeck et al. 2019), since it characterizes overweight and obesity independently from factors, such as age, sex, ethnicity, and body composition, among others (World Health Organization 2022). This suggests that a true reflection of this complex multifactorial chronic disorder can only be captured by identifying different “obesities”, based on metabolic subgroups with similar metabolic characteristics (Blundell et al. 2014).

The application of personalized nutrition for weight management based on an individual’s postprandial metabolic phenotype might be useful, since studies have shown that PPGR is associated with appetite control (Astrup and Hjorth 2018), and an increased accumulation of fat is associated with insulin resistance in different organs (Chia et al. 2018; Trouwborst et al. 2018). Although research has provided evidence for the potential of personalized nutrition in obesity management, they usually focus on short-term weight loss. There is still limited research in the area of personalized nutrition, incorporating deep phenotyping approaches for a more holistic phenotyping strategy, aiming for effective long-term weight management.

2 AIM OF THE THESIS

Research has shown that mechanisms influencing the variability of postprandial metabolic response go beyond mere human physiology. This raises the question of which factors might influence the observed inter-individual variability of the postprandial state. In this work, a human intervention study was developed as an experimental approach to assess the postprandial metabolic phenotype of people with obesity. Additionally, cross-sectional data were analyzed and a systematic literature review was conducted to evaluate two possible influencing factors shaping the PPGR: the genotype and the diet (Figure 4).

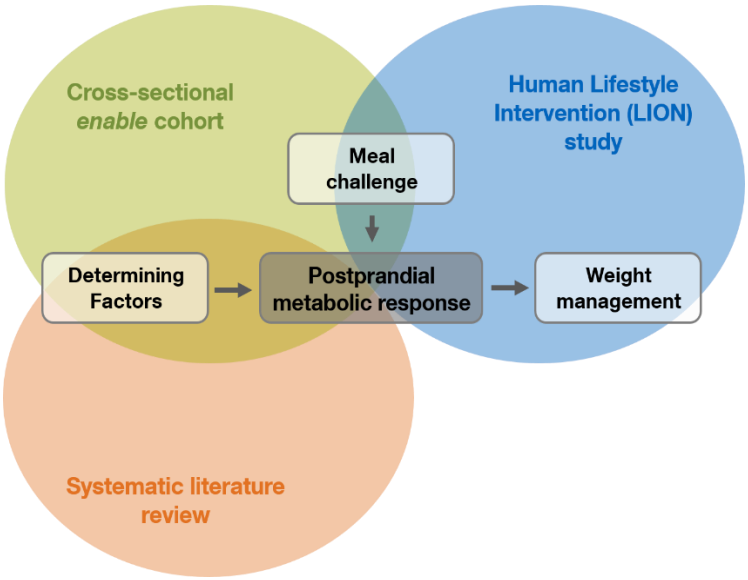

Figure 4 | Schematic overview of the methods and aims of this work.

These different methods are applied to address the following research questions (Figure 4).

1) Implementation of the Lifestyle Intervention (LION) study to investigate the postprandial metabolic response to different meal challenges as a phenotyping tool and as a predictor for the efficacy of weight management in people with obesity.
2) Investigation of associations between the habitual diet and the PPGR in the cross-sectional *enable* cohort.
3) Overview of associations between the genotype and the PPGR by a systematic literature review.

Overall, this work describes the data collection for the assessment of the inter-individual variability of PPGR and evaluates factors affecting the PPGR. Finally, this work seeks to derive suggestions for both (a) the clinical implementation of individualized weight management interventions for people living with obesity and (b) future research on the optimization of evidence-based personalized nutrition.

3 METHODS

3.1 HUMAN STUDY - LION STUDY

The LION study was established to evaluate the effect of two different diets (low carbohydrate and low fat) and two digital tools (smartphone application (app) and newsletter) on long-term weight maintenance 12 months after weight loss. Weight change after 15 months of study participation is the primary outcome, whereas changes in further assessed parameters are secondary outcomes. There is interest in research to identify inter-individual phenotypical variations based on a deep phenotyping approach (including data collection such as anthropometric, metabolic, genetic, clinical, lifestyle, psychological, and environmental data) and to evaluate how phenotypical variations might predict the outcome of weight management interventions for people living with obesity. The here presented exploratory analysis was performed to phenotype people with obesity, based on their PPGR.

3.1.1 LION - STUDY DESIGN AND STUDY POPULATION

The LION study is a single-site study designed as a 2 × 2 factorial RCT including four study steps: (I) screening and baseline phenotyping, (II) weight loss intervention, (III) weight maintenance intervention, and (IV) the follow-up period (Figure 5). Recruitment took place between July 2019 and October 2021 at the Institute for Nutritional Medicine at the University Hospital “Klinikum rechts der Isar” at the Technical University of Munich, Germany. This study is performed following the ethical principles of the Declaration of Helsinki. The study protocol was approved by the ethics review committee of the School of Medicine, Technical University of Munich (vote: 69/19 S). The trial was registered at clinicaltrials.gov (NCT04023942) and the German Clinical Trials Register (DRKS00017819).

Healthy adults aged between 18 - 65 years, with a BMI between 30.0 - 39.9 kg/m², that own a smartphone were considered eligible for participation. Eligibility was verified both during a telephone-based screening interview and during the first face-to-face visit according to defined exclusion criteria. Written informed consent was provided before any measurements were conducted.

3.1.2 LION - CURRICULUM AND DATA COLLECTION

Participants attend seven face-to-face visits (marked as blue arrows) during the study (Figure 5). All measures were performed after an 8-h overnight fast, with no rigorous physical activity in the past 12 hours. The LION study starts with two consecutive baseline face-to-face visits

(V1A and V1B), where clinical examinations (e.g., anthropometry, vital parameters, blood samples) and meal challenges (e.g., OGTT) were performed.

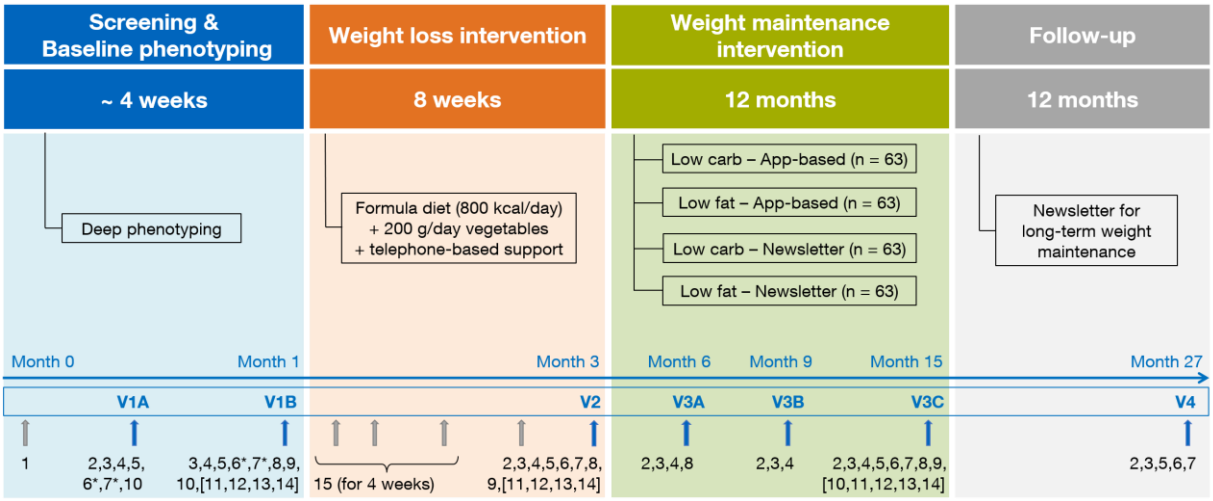

Figure 5 | Updated version of the schematic overview of the study design.
Modified according to (Reik and Holzapfel 2020). Abbreviation: carb, carbohydrate. Numbers refer to measurements taking place during the correspondent visit: [1]Screening questionnaire, [2]Anthropometry, [3]Blood parameters/samples, [4]Questionnaires/protocols, [5]Vital parameters, [6]Resting metabolic rate, [7]Hand strength measurement, [8]Urine parameters/samples, [11]Collection of saliva samples, [10]Meal challenges, [11]Collection of fecal samples, [12]Magnetic resonance imaging, [13]Physical strength, motor function, and body static condition, [14]Electrocardiogram, [15]Continuous glucose measurement. The time point of examinations marked with * is variable since it depends on the randomization order of meal challenges. Examinations marked by square brackets are optional (subgroup analysis).

The following weight loss intervention (step II) encompassed an 8-week formula diet (Itrim, Itrim Sverige AB, Sweden) providing 800 kcal/day. During this period, an additional daily intake of 200 g of vegetables was allowed and participants were closely supervised by the study team. A CGM (FreeStyle Libre Pro, Abbott Diabetes Care Inc., USA) was applied in a subcohort for the first 4 weeks of intervention. Additionally, participants were asked to record their daily dietary intake in a food diary. A face-to-face visit (V2) completed the weight loss intervention, in which some measurements were repeated. If a weight loss > 4 kg was achieved, the participant proceeded with step III.

For the 12-month weight maintenance intervention (step III), participants were randomly assigned to one of four possible weight maintenance programs: low carbohydrate & app-based group, low carbohydrate & newsletter-based group, low fat & app-based group, or low fat & newsletter-based group. The recommended total daily energy intake was individually defined based on the resting metabolic rate (RMR) measured after weight loss (V2) and the physical activity level (PAL) determined by self-assessment. Participants were instructed to follow dietary guidelines specific to their corresponding intervention arm: (a) low carbohydrate diet: the total daily energy intake should be covered with 30 energy percent (E%) from carbohydrates, 20 E% from protein, and 50 E% from fat; (b) low fat diet: 25 E% from fat, 20

E% from protein, and 55 E% from carbohydrate. Participants were instructed to keep a dietary record during this period. Either an app or a newsletter was applied as a digital counseling tool to support nutritional guidance. The app-based intervention includes regular contact with a nutritional coach in the chat function of the app (Oviva, Oviva AG, Switzerland). These contacts occurred in a decreasing frequency (weekly in the first 3 months, every 2 weeks in the following 6 months, and once a month in the last 3 months). Participants assigned to the newsletter-based intervention received newsletters via email, at the same frequency as "contacts" took place in the app-based group. During the weight maintenance intervention, visits take place when participants reach the third month (V3A), the sixth month (V3B), and the twelfth month (V3C) mark of the intervention.

During follow-up (step IV), newsletters designed for additional support of long-term weight maintenance are sent quarterly per mail. The study is finalized by the conclusion of a final visit (V4) after 12 months of follow-up.

Anthropometric measurements were performed with light clothing, without shoes, and with an empty bladder. Body composition was assessed by bioelectrical impedance analysis (BC-418MA; Tanita Europe B.V., Netherlands). Height was measured with a stadiometer (Seca 214, Seca GmbH & Co. KG, Germany). Waist and hip circumferences were assessed using a non-stretch measuring tape in a standing position. Blood pressure was measured with a blood pressure monitor with a cuff around the upper arm (BM 60 or BM 70, BEURER GmbH, Germany; M400 Intelli IT, OMRON Medizintechnik Handelsgesellschaft mbH, Germany).

The OGTT drink provided 82.5 g of ≥ 99.5 % α-d (+)-glucose monohydrate Ph. Eur. (Carl Roth GmbH & Co. KG, Germany), corresponding to 75 g glucose in 300 ml of tap water. Blood was drawn from all participants in the fasted state and at 30 min intervals for a total of 2 hours. Collected blood was stored at room temperature until sent to an external lab (SYNLAB Medizinisches Versorgungszentrum Labor München Zentrum GbR, Munich, Germany) for the analysis of routine parameters (at baseline) and the assessment of postprandial glucose, insulin, and TAG levels.

3.1.3 LION - STATISTICAL ANALYSIS

Statistical analysis was conducted with the integrated development environment RStudio (RStudio version 1.3.959, Boston, USA) alongside the R programming language version 4.2.1. (R Core Team, 2022, http://www.r-project.org).

An exploratory analysis of the LION cohort was performed to describe baseline characteristics and changes in anthropometric measures after weight loss, as well as to evaluate the PPGR

to an OGTT. For this purpose, data from 272 participants that finished the 8-week weight loss intervention were considered.

Data on age, BMI, anthropometrics, and vital parameters were analyzed to describe baseline characteristics (mean ± SD) of the total cohort and after stratification by sex. The PPGR was defined as incremental glucose AUC and was calculated based on the trapezoidal rule, considering the lowest glucose concentration as a baseline value. The coefficient of variation of the PPGR was calculated as the ratio between the standard deviation and the mean of glucose AUC. Changes in anthropometric measures after weight loss intervention (delta, Δ), such as weight, BMI, fat mass, fat-free mass, waist circumference (WC), and hip circumference (HC) were calculated as follows: measured value at baseline subtracted by measured value at visit V2. Clustering of the postprandial glucose trajectories was performed based on longitudinal k-means clustering (Genolini et al. 2015) considering the classification criteria by Calinski and Harabasz (Calinski and Harabasz 1974).

3.2 Cross-sectional data analysis - Habitual diet and postprandial glucose response

Cross-sectional data analysis was performed to assess the potential effects of the habitual diet on the PPGR. This work was conducted with data from the *enable* cohort, which includes healthy participants from different age groups, and was established within the *enable* competence cluster of nutrition research as a phenotyping platform (Brandl et al. 2020). In this chapter, data collection and definitions included in this analysis are briefly summarized.

3.2.1 *enable* cohort - Study design and study population

Recruitment took place between 2016 and 2018 at the Human Study Center of the Central Institute of Nutrition and Food in Freising and at the Institute for Biomedicine of Aging in Nuremberg, Germany. Participants attended two face-to-face visits, in which clinical examinations (e.g., anthropometry, blood samples) and an OGTT were performed. Furthermore, different questionnaires (e.g., nutritional behavior) were completed during the visits.

The study protocol follows ethical principles of the Declaration of Helsinki and has been approved by the local ethics review committee of the School of Medicine, Technical University of Munich (vote: 452/15) and of the Friedrich-Alexander-University Erlangen-Nuremberg (vote: 291/15B). The trial was registered in the German Clinical Trials Register (DRKS00009797).

To evaluate the research question, data from 459 healthy white adults with a BMI between 18.5 - 35.0 kg/m² were considered. In total, 94 young adults (18 - 25 years), 205 middle-aged adults (40 - 65 years), and 160 older adults (75 - 85 years) were included for analysis. Eligibility was verified in a telephone-based screening interview and written informed consent was provided before any measurements were conducted. Exclusion criteria were defined as any severe medical condition (e.g., cardiovascular, liver, kidney, lung diseases; untreated endocrine diseases; autoimmune diseases; chronic infections/inflammations; psychological or neurological disease; diabetes mellitus; cancer; stomach ulcer), blood donation or transfusion performed in the past 3 months, immobility, tobacco use, weight loss (> 5 %) in the past 3 months, and participation in intervention studies.

3.2.2 *enable* COHORT - CURRICULUM AND DATA COLLECTION

In visit 1, body composition was assessed by bioelectrical impedance analysis (Seca mBCA 515; Seca GmbH & Co. KG, Germany), height was measured with a stadiometer, and WC and HC were measured with a measuring tape. Systolic and diastolic blood pressure were assessed by a blood pressure monitor (Omron M8 comfort, OMRON, Germany). Finally, blood was drawn in the fasted state by venipuncture and was sent to an external lab (SYNLAB Medizinisches Versorgungszentrum Labor München Zentrum GbR, Munich, Germany) for the analysis of parameters usually measured in routine clinical care.

In visit 2, an OGTT was performed in the morning after a 12-hour fast. Blood was drawn before and 30, 60, 90, 120, 180, and 240 minutes after glucose load (drink providing 75 g glucose in 300 ml boiled tap water). Plasma glucose levels were measured by a plasma-calibrated rapid tester (HemoCue® Glucose 201+ System, HemoCue AB, Sweden).

The PPGR as the outcome variable was defined as glucose incremental AUC, calculated using the trapezoidal rule with the lowest postprandial glucose concentration as the baseline value. Dietary data were considered independent variables and were collected through a web-based food frequency questionnaire (FFQ) (Nöthlings et al. 2007) and two 24-hour food lists (Freese et al. 2014). Dietary data were used to estimate the habitual diet, which was defined as the usual intake of food groups (Mitry et al. 2019) and nutrients. For the calculation of nutrient intake, the amount of the consumed food groups was linked to the German food composition database (Bundeslebensmittelschlüssel, version 3.02.). Data were further used to estimate the modified Alternate healthy eating index (AHEI) (Wawro et al. 2020), which evaluates the "health value" of a dietary pattern. For the calculation of the AHEI score, the total intake of ten available food components is assigned to a value ranging from 0 to 10. According to this

rating system, the AHEI score can reach a maximum of 100 points, with a higher score corresponding to greater adherence to a “healthier” dietary pattern.

3.2.3 *enable* COHORT - STATISTICAL ANALYSIS

Analysis was performed with the integrated development environment RStudio (RStudio version 1.3.959, Boston, USA), used alongside R programming language version 4.0.0 (R Core Team, 2010, http://www.r-project.org).

Linear regression models were fitted to evaluate cross-sectional associations of habitual diet with glucose AUC (adjusted for total energy intake, BMI, sex, and age). Furthermore, a multivariate stepwise regression model with forward and backward selection was conducted, in which total energy intake, BMI, sex, and age were chosen as *a priori* adjustment predictors. Diet-related variables (e.g., intake of macronutrients, fiber, and food groups) were chosen as facultative predictors, that only enter the model if it leads to an improved model, characterized by the Akaike information criteria (AIC) value (Akaike 1974). Predictor variables were scaled so that estimates refer to the average change of the outcome variable (glucose AUC) when the corresponding predictor variable is increased by one standard deviation. Winsorization was performed (with a percentile limit set at 0.01) to reduce the effects of under- and over reporters.

A post hoc cluster analysis, based on longitudinal k-means clustering (Genolini et al. 2015) and previously published clustering criteria (Calinski and Harabasz 1974), was conducted to identify subgroups with homogenous postprandial glucose trajectories. To assess associations between the habitual diet and the cluster assignment, multinomial logistic regression, as well as stepwise regression models were applied.

3.3 SYSTEMATIC REVIEW - GENOTYPE AND POSTPRANDIAL GLUCOSE RESPONSE

A systematic review was performed to assess the potential contributions of the genotype on the variations of PPGR. To summarize current evidence, articles investigating the effects of SNPs on the glucose AUC after an OGTT were systematically searched. The systematic review was performed based on the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) (Page et al. 2021) and was registered in the International Prospective Register for Systematic Reviews (PROSPERO, registration number CRD42021231203).

3.3.1 SEARCH STRATEGY AND SELECTION CRITERIA

The literature search started in January 2021 and was conducted in three different electronic databases (Web of Science, Embase, PubMed). The search strategy defined search terms in the categories "genetic", "intervention", and "outcome" (Table 2), which were combined using the Boolean operator "AND". Detailed search terms within each category are listed in Table 2 and were combined in the search inquiry with the Boolean operator "OR".

Table 2 | Search strategy for the identification of relevant articles.

genetic		intervention		outcome
"polymorphism" OR *"polymorphisms"* OR *"variant"* OR *"variants"* OR *"genotype"* OR *"genotypes"* OR *"SNP"* OR *"SNPs"* OR *"gene locus"* OR *"gene loci"* OR *"genetic locus"* OR *"genetic loci"*	AND	*"OGTT"* OR *"challenge"* OR *"challenges"* OR *"oral"* OR *"hour"* OR *"tolerance test"* OR *"tolerance tests"* OR *"fasting"* OR *"glucose tolerance"*	AND	*"glucose"* OR *"glycemic"* OR *"glycaemic"* OR *"postprandial"* OR *"response"* OR *"responses"*

The inclusion criteria were determined before the literature search and the review process. Articles were required to address studies with human participants, published in English since the year 2000. Therefore, filters such as "humans only", "since 2000", and "English language" were applied directly to the search query in the database, if possible. GWAS were excluded from the review process. Additional inclusion criteria were defined based on the PICO principle (Booth and Brice 2004), and were considered for the identification of suitable articles:

- **P**opulation: Healthy participants older than 18 years. Analysis of participants with severe diseases (e.g., diabetes), as well as of pregnant or breastfeeding women, and children were excluded.
- **I**ntervention: OGTT.
- **C**ontrol: Results of non-risk allele carriers of any investigated SNP.
- **O**utcome: PPGR, defined as glucose AUC.

Identified articles were imported in the reference management tool EndNote X9.3.3 (Clarivate, Philadelphia, USA), duplicates were removed, and were further screened independently by two reviewers for their eligibility. If there was disagreement on the eligibility of a publication, further two reviewers were consulted for consensus on a final decision. Additional publications not returned by the initial database search were manually identified by checking

the citations of eligible articles. Finally, the full text of all suitable articles was retrieved for further evaluation.

3.3.2 Data extraction and quality assessment

Data extraction was performed independently by two reviewers using an excel sheet (Microsoft Excel 2016; Microsoft Corporation, Redmond, USA). Data regarding the first author's name, publication year, study name, description of the study population, sample size (for calculation of glucose AUC), intervention time, genes of interest, analyzed SNPs, statistical results (*p*-value) of the association between SNPs and glucose AUC, and further relevant details were extracted and were finally compared between reviewers to clarify discrepancies. The linkage disequilibrium has been calculated using the genome browser Ensembl (Howe et al. 2021). A quality assessment tool for the evaluation of genetic association studies was applied to estimate the quality of the eligible articles (Campbell and Rudan 2002). Based on this rating system, studies were rated as high (5.5 to 11 points), intermediate (0 to 5 points), or low quality (-11 to -0.5 points).

The results of this systematic review were summarized and presented by narrative synthesis.

4 RESULTS

This chapter briefly summarizes the results of the manuscripts and data analyses that are the subject of this work.

4.1 THE LION STUDY

4.1.1 IMPLEMENTATION OF A HUMAN INTERVENTION STUDY

Title: 'Randomized Controlled Lifestyle Intervention (LION) Study for Weight Loss and Maintenance in Adults with Obesity – Design and Methods.'

Authors: **Anna Reik**, Christina Holzapfel.

Access: Frontiers in Nutrition 2020; 7:586985; doi: 10.3389/fnut.2020.586985; Open Access article available at: https://www.frontiersin.org/articles/10.3389/fnut.2020.586985/full.

Summary:

Weight loss and maintenance are challenging and heterogeneous results with high inter-individual variability are observed in intervention studies. These findings suggest that established weight management guidelines, based on a "one-size-fits-all" approach, may not be effective for everyone. Therefore, there is a rising interest to identify factors influencing inter-individual differences in weight loss and maintenance, moving toward personalized strategies for weight management that are effective in the long term. The LION study is a 2 × 2 factorial RCT, primarily designed to evaluate the efficacy of two diets (low carbohydrate vs. low fat) and two digital tools (app vs. newsletter) on long-term weight maintenance, and secondly to identify predictors (e.g., genetic, epigenetic, physiological, psychological, microbiological) for successful weight management.

Participants with a BMI between 30.0 - 39.9 kg/m², aged 18 - 65 years, in ownership of a smartphone, and without severe diseases were recruited between July 2019 and October 2021. After baseline phenotyping, participants follow a formula-based low-calorie diet for 8 weeks as a weight loss intervention (Figure 5). Based on power calculations, 252 participants are needed to be randomized into one of the four intervention groups (low carbohydrate & app, low carbohydrate & newsletter, low fat & app, low fat & newsletter) for the 12-month weight maintenance intervention (Figure 5). The LION study is concluded after another 12 months of follow-up (Figure 5). During the approximately 27-month period of enrollment, seven face-to-face visits with repeated measurements take place. The LION study incorporates methodological diversity, allowing an in-depth evaluation of the complex nature

of obesity and weight management. Results will provide indications for successful weight management interventions and give insights into a personalized approach to the treatment of people living with obesity.

Personal contribution: **Anna Reik** was involved in the development of the study design and the conceptualization of the study protocol. **Anna Reik** is currently involved in the study management and is further responsible for data collection and data management. **Anna Reik** wrote the manuscript and prepared tables and figures for the published article.

4.1.2 Exploratory analysis of the LION cohort – Postprandial glucose response as a phenotyping tool

Working title: 'The association between postprandial metabolic response to meal challenges and weight loss in people with obesity: an exploratory analysis of the lifestyle intervention (LION) study.'

Authors: **Anna Reik** *et al.*

Access: Article in preparation.

Summary of preliminary findings:

Among 978 registrations for participation and 493 telephone-based screenings, 347 candidates were determined to be potentially qualified and invited to attend the first face-to-face visit (Figure 6). A total of 318 participants were deemed eligible for participation and started the formula diet for weight loss (Figure 6). The weight loss step was concluded in December 2021, and after taking drop-out and failure (16.9 %) into account, 272 participants were randomized to one of the weight maintenance intervention arms: (a) low carbohydrate & app (n = 69), (b) low carbohydrate & newsletter (n = 68), (c) low fat & app (n = 67), or (d) low fat & newsletter (n = 68) (Figure 6). The last V3A and V3B visits were performed by the end of March 2022 and by the end of July 2022, respectively. Since the LION study is ongoing, data collection for visits V3C, and V4 is still in progress.

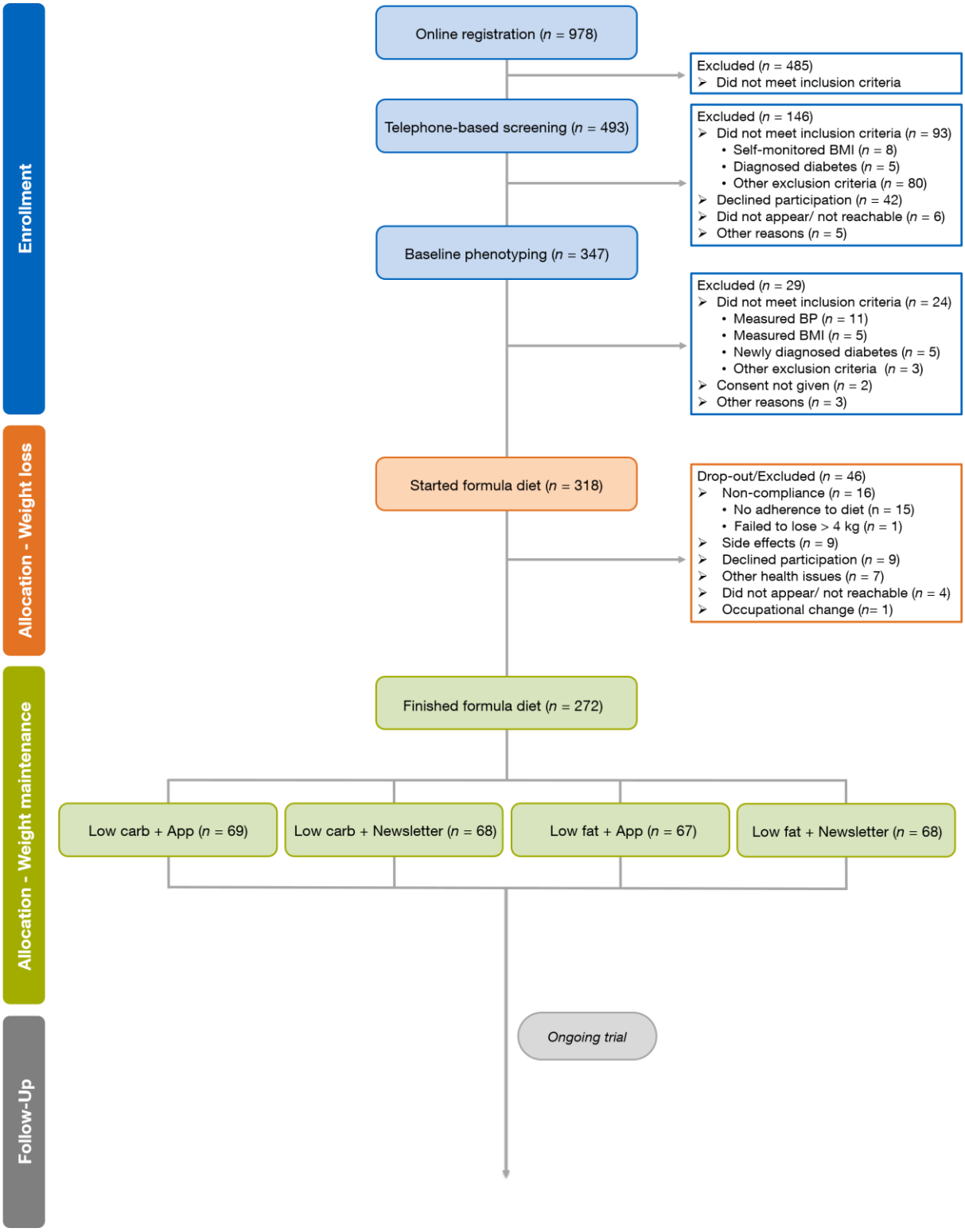

Figure 6 | CONSORT diagram for the LION study.

Baseline characteristics of the study cohort are presented in Table 3. A total of 272 participants were assigned to one of the weight maintenance interventions, of which 64 % are female. The total cohort had a mean age of 45 ± 11 years and a mean BMI of 34.55 ± 2.85 kg/m² at baseline (Table 3).

Table 3 | Baseline characteristics of participants of the LION study.

Variable[1]	Total, *N* = 272[2]	Male, *n* = 98[2]	Female, *n* = 174[2]
Age [years]	45 (± 11)	43 (± 11)	47 (± 11)
Weight [kg]	103 (± 14)	114 (± 13)	96 (± 10)
BMI [kg/m²]	34.55 (± 2.85)	34.63 (± 2.94)	34.51 (± 2.80)
Fat mass [%]	40 (± 7)	31 (± 4)	44 (± 3)
Fat mass [kg]	40 (± 8)	36 (± 7)	43 (± 7)
Fat-free mass [kg]	62 (± 13)	78 (± 8)	53 (± 5)
WC [cm]	105 (± 11)	113 (± 9)	100 (± 9)
HC [cm]	119 (± 8)	116 (± 7)	121 (± 8)
WHR	0.88 (± 0.10)	0.98 (± 0.07)	0.83 (± 0.08)
Systolic BP [mmHg]	125 (± 13)	130 (± 10)	122 (± 13)
Diastolic BP [mmHg]	86 (± 8)	88 (± 7)	85 (± 8)
Glucose [mg/dl]	90 (± 9)	92 (± 8)	89 (± 10)
HbA1C [%]	5.29 (± 0.39)	5.25 (± 0.38)	5.32 (± 0.40)
Insulin [µU/ml]	12 (± 11)	15 (± 15)	11 (± 8)
Total cholesterol [mg/dl]	207 (± 37)	204 (± 33)	208 (± 39)
TAG mg/dl]	130 (± 61)	153 (± 77)	117 (± 45)
HDL-C [mg/dl]	53 (± 13)	45 (± 9)	57 (± 13)
LDL-C [mg/dl]	144 (± 37)	146 (± 33)	143 (± 38)

[1]Abbreviations: BMI, body mass index; BP, blood pressure; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol; WC, waist circumference; WHR, waist-to-hip ratio. [2]Mean (± SD).

Out of the 272 participants that completed the weight loss intervention, a total of 161 participants (59 males and 102 females) provided data for the PPGR after OGTT. The mean postprandial blood glucose trajectory during the OGTT of male and female participants is presented in Figure 7A. The peak of mean postprandial glucose concentration is reached 60 min after glucose drink consumption. Inter-individual variations of the PPGR to an OGTT can be observed in the LION cohort (Figure 7B) and is evident by the calculated coefficient of variation of 53.11 %, which is higher than the coefficient of variation of baseline glucose (16.67 %).

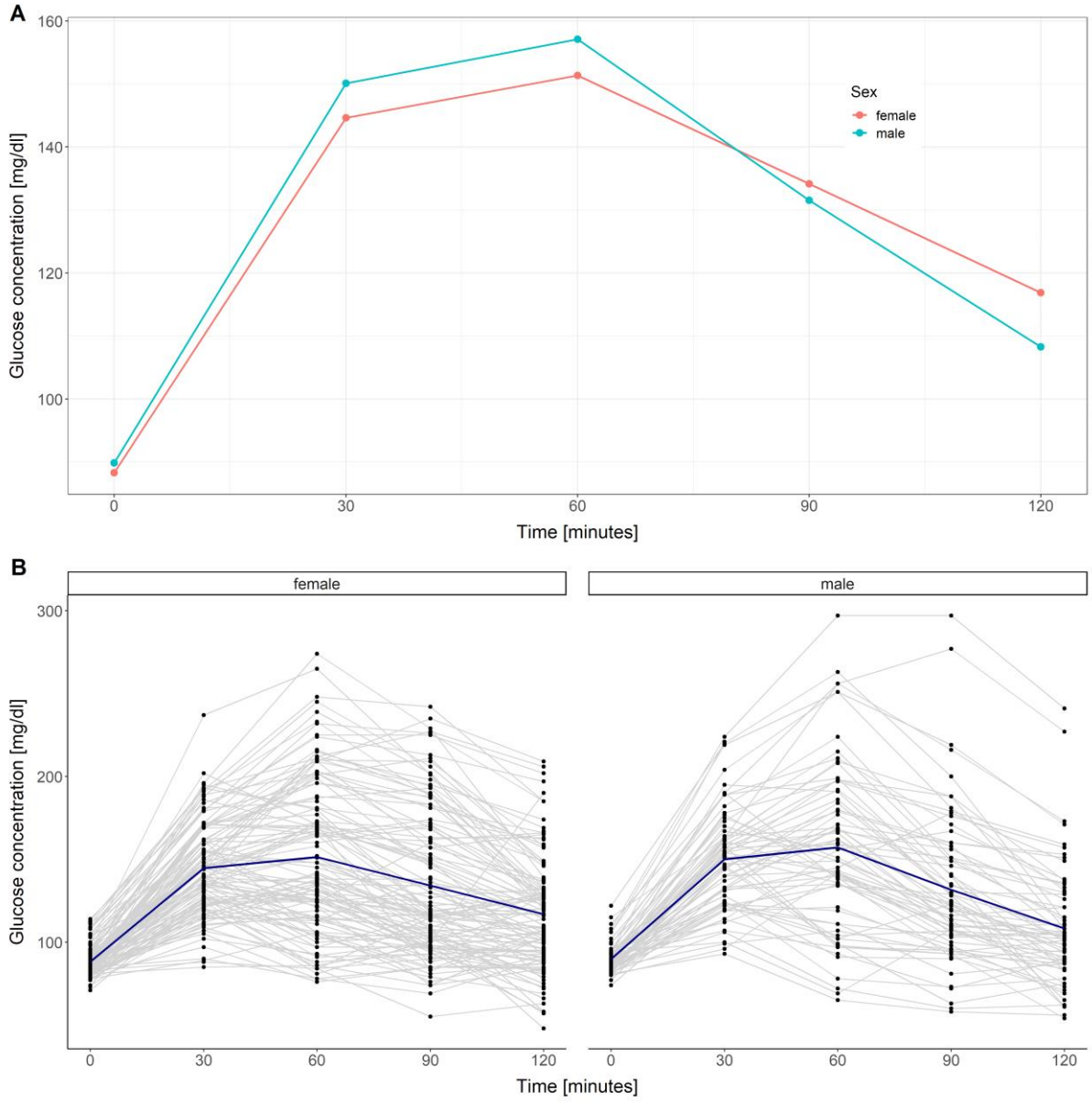

Figure 7 | Postprandial glucose trajectories after an OGTT.
(A) Mean postprandial glucose trajectories for male and female participants. (B) Individual postprandial glucose trajectories with plotted dots for blood glucose levels at each time point (grey) with the mean blood glucose concentrations (blue) after drink consumption.

Cluster analysis shows two types of postprandial glucose trajectories (Figure 8). The mean postprandial glucose level of cluster A (red) reaches its peak around 30 min and rises lower (< 150 mg/dl) (data not shown). In contrast, cluster B (blue) reveals a delayed postprandial glucose peak at 60 min at higher levels (> 150 mg/dl) (data not shown).

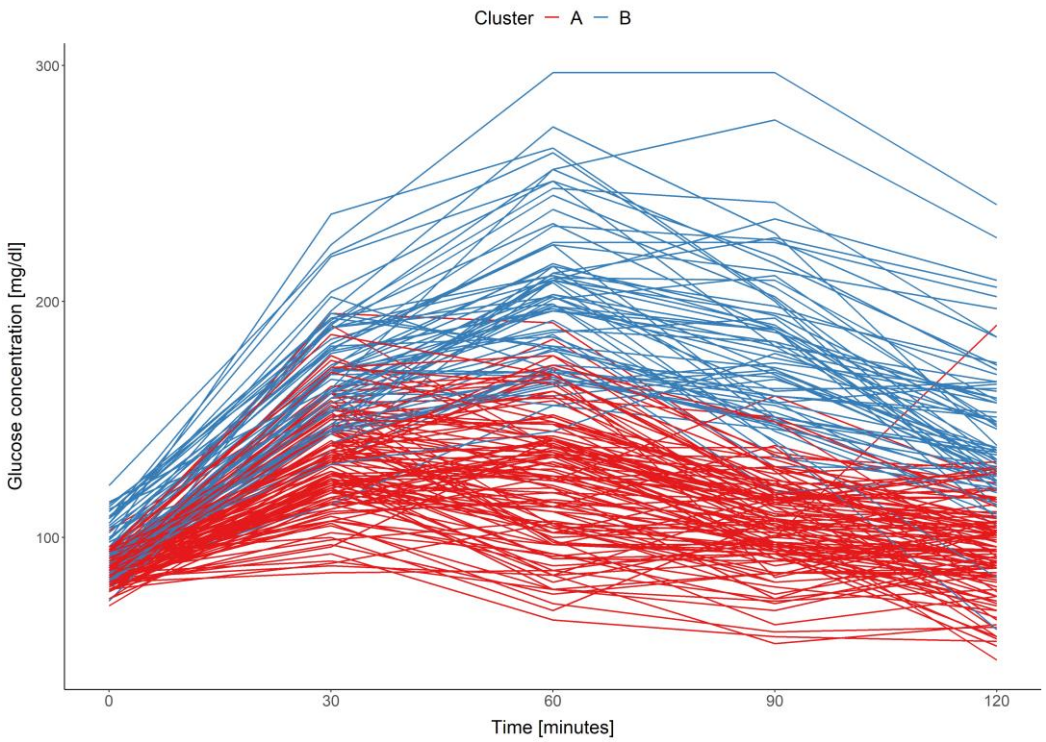

Figure 8 | Postprandial blood glucose trajectories with color-coded cluster assignment.

Table 4 summarizes changes in selected anthropometric parameters after the weight loss intervention. Overall, a mean weight loss of 11.8 ± 3.5 kg was achieved by the total cohort after 8 weeks of formula diet. Fat mass accounted for the larger share of lost weight in comparison to fat-free mass (8.16 ± 2.50 kg vs. 3.65 ± 2.18 kg, respectively). Males lost by average 3.8 kg more body weight during this period in comparison to females but lost fat-free mass to a greater extent (5.24 ± 2.03 kg and 2.76 ± 1.71 kg, respectively). Male participants had a greater reduction of fat mass localized in the abdomen since the decrease of WC was higher compared to HC (11.2 ± 3.9 kg vs. 7.5 ± 3.7 kg).

Table 4 | Changes (Δ) of anthropometric parameters after weight loss intervention.

Variable[1]	Total, *N* = 272[2]	Male, *n* = 98[2]	Female, *n* = 174[2]
Δ Weight [kg]	11.8 (± 3.5)	14.2 (± 3.7)	10.4 (± 2.6)
Weight loss [%]	11.49 (± 2.99)	12.55 (± 3.35)	10.89 (± 2.59)
Δ BMI [kg/m²]	3.95 (± 1.01)	4.32 (± 1.11)	3.74 (± 0.88)
Δ Fat mass [*pp*]	4.05 (± 2.16)	4.71 (± 2.02)	3.68 (± 2.15)
Δ Fat mass [kg]	8.16 (± 2.50)	8.98 (± 2.57)	7.72 (± 2.35)
Δ Fat-free mass [kg]	3.65 (± 2.18)	5.24 (± 2.03)	2.76 (± 1.71)
Δ WC [cm]	8.7 (± 5.0)	11.2 (± 3.9)	7.3 (± 5.1)
Δ HC [cm]	7.4 (± 4.5)	7.5 (± 3.7)	7.4 (± 4.9)

[1]Abbreviations: BMI, body mass index; HC, hip circumference; *pp*, percentage points; WC, waist circumference. [2]Mean (± SD).

Personal contribution: **Anna Reik** was involved in conceiving and designing the research question. **Anna Reik** processed the experimental data. **Anna Reik** conducted the statistical analysis. Finally, **Anna Reik** is preparing tables and figures.

4.2 Association between the habitual diet and the postprandial glucose response to an OGTT

Title: 'Association between habitual diet and the postprandial glucose response – an enable study.'

Authors: **Anna Reik**, Beate Brandl, Gunther Schauberger, Nina Wawro, Jakob Linseisen, Thomas Skurk, Dorothee Volkert, Hans Hauner, Christina Holzapfel.

Access: Molecular Nutrition and Food Research 2022; e2200110; doi: 10.1002/mnfr.202200110; Open Access article available from: https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202200110.

Summary of findings:

This cross-sectional analysis aimed to evaluate whether the habitual diet is associated with the PPGR (glucose AUC) after an OGTT.

The *enable* cohort (*N* = 459, 50 % women, mean age 55 ± 21 years, mean BMI 26 ± 5 kg/m²) consisted of participants from three different age groups (*n* = 94 young adults aged 18 - 25 years, *n* = 205 middle-aged adults aged 40 - 65 years, and *n* = 160 older adults aged 75 - 85 years). Fasting blood glucose was similar in each age group, but postprandial glucose trajectories differed between and within age groups. Similarly, generation-specific food preferences were observed. Univariate linear regression models revealed that the intake of cereals and cereal products is negatively associated with glucose AUC for the total cohort (adjusted estimate -1,009.28, 95 % CI -1,659.73 to -358.84, adjusted $p = 0.002$). Despite pulses and legumes as well as meat and meat products being positively associated with glucose AUC in middle-aged adults (adjusted $p = 0.002$), no other diet-related parameter was associated with glucose AUC in the other age groups. Multivariate prediction models based on the stepwise regression method revealed a different set of predictors for glucose AUC for the total cohort and in each age group. The usual intake of cereals and cereal products stands out since an increased consumption significantly predicts a reduced glucose AUC in the total cohort (adjusted estimate -748.61, 95 % CI -1,424.27 to -72.94, adjusted $p = 0.030$), young (adjusted estimate -1,504.48, 95 % CI -2,722.99 to -285.98, adjusted $p = 0.016$) and older adults (adjusted estimate -1,580.36, 95 % CI -2,779.52 to -381.19, adjusted $p = 0.010$).

Overall, no dietary parameter equally predicts glucose AUC in all three age groups. Food groups or nutrients explained up to 9 % of the observed variation in glucose AUC, whereby differences regarding the variability are also dependent on the age group.

In an exploratory analysis, the cohort was grouped based on the shape of their postprandial blood glucose trajectory. Three clusters were identified, of which cluster A is characterized by the lowest "metabolic risk" (e.g., lowest BMI and fasting blood glucose) and the lowest glucose AUC. In contrast, cluster C includes participants with the highest "metabolic risk" and the highest glucose AUC. Multinomial logistic regression models show that a higher intake of carbohydrates, dairy products, cereals and cereal products, and condiments and sauces leads to a greater odds ratio for assignment to cluster A or B. Stepwise regression models have derived the same results for the carbohydrate intake and the consumption of cereals and cereal products.

In conclusion, these findings suggest that the overall habitual diet does not seem to have a major impact on PPGR, since the usual carbohydrate intake and the usual intake of cereals and cereal products were the only parameters found to be persistently associated with glucose AUC. Further, there are age-specific differences regarding the association between habitual diet and postprandial metabolism. Further research is needed to clarify how the habitual diet contributes to postprandial metabolism and to investigate if diet can be optimized based on an individual's age and PPGR.

Personal contribution: **Anna Reik** was involved in conceiving and designing the research question. **Anna Reik** processed the experimental data. **Anna Reik** conducted the statistical analysis under the guidance of co-authors. Finally, **Anna Reik** prepared tables and figures and wrote the manuscript.

4.3 Association between the genotype and the postprandial glucose response to an OGTT

Title: 'Association between genotype and the glycemic response to an oral glucose tolerance test: a systematic review.'

Authors: Sandra Bayer, **Anna Reik**, Lena von Hesler, Hans Hauner, Christina Holzapfel.

Access: Article submitted for publication in the Journal Molecular Nutrition and Food Research.

Summary of findings:

This systematic review aimed to give an overview of current evidence regarding the association between SNPs and PPGR (glucose AUC) after an OGTT.

A total of 33,040 articles were identified through an initial database search, and further 179 articles were identified by a manual search of the reference lists. A total of 139 articles were deemed eligible after screening. However, to increase reproducibility and reduce the presentation of single findings, only articles including SNPs studied in more than 3 articles were considered for narrative synthesis. In total, 49 articles investigating 53 SNPs in 13 gene loci were included for final evaluation. The most investigated gene loci included *TCF7L2*, *PPARγ, and potassium inwardly rectifying channel subfamily J member 11 (KCNJ11)*. Results are heterogeneous, showing either no association or non-replicable single findings for an association between the investigated SNPs and glucose AUC.

In conclusion, robust evidence for an association between SNPs and glucose AUC cannot be confirmed in this review. Subsequently, it is not evident that the genotype has any clinical implications for postprandial glucose metabolism. Polygenetic scores might be a more promising tool to investigate genetic contributions to postprandial metabolism and should be addressed in the future.

Personal contribution: **Anna Reik** provided support concerning the definition of the search strategy, inclusion criteria, and outcome. **Anna Reik** further provided support in the reference-based identification of additional articles, the retrieval of full-text articles, and the final decision on the eligibility of identified publications. **Anna Reik** wrote the introduction of the manuscript and was involved in drafting the tables for the published article. Finally, **Anna Reik** revised the manuscript and commented on the publication as a co-author.

5 DISCUSSION

5.1 IMPLEMENTATION OF THE LION STUDY - LESSONS LEARNED

The LION study provides a unique multi-dimensional set of data for detailed phenotyping of people living with obesity (Reik and Holzapfel 2020). The results of this study will provide a better tool to evaluate the metabolic phenotype and further address weight regulation as an expression of metabolic flexibility (Reik and Holzapfel 2020), in which weight gain or weight loss presents a form of metabolic response. In this context, multi-faceted data, collected at baseline, are evaluated to advance the understanding of obesity-related phenotypes, allowing predictions of weight management interventions. Further, the effect of either a low fat or a low carbohydrate diet on weight maintenance is tested to elucidate if different postprandial metabolic phenotypes respond differently to these diets. However, the postprandial metabolic phenotype might depict just one piece of the puzzle that explains the etiology of obesity. By integrating further information from environmental, sociocultural, behavioral, physiological, genetic, and epigenetic factors as contributors to obesity, results from the LION study might help to pave the way for a more effective and personalized prevention or treatment of obesity.

However, some methodological drawbacks of the LION study should be addressed. First, the meal challenges for the assessment of the postprandial metabolic phenotype were only planned as a baseline measurement (Reik and Holzapfel 2020). Therefore, our data will not allow the evaluation of the dynamics of postprandial metabolism by determining changes after the weight management interventions, which would have been interesting since the metabolism can adapt to environmental changes (Lépine et al. 2022). To address this, an amendment of the study protocol has been submitted to the ethics committee in September 2021. After acceptance in January 2022, meal challenges were introduced at visit V3C as an optional measure, allowing a further evaluation of the postprandial metabolic response after the weight maintenance intervention.

Furthermore, recruitment and data collection took place over a long period, in which the interplay between population and environment can have major effects on future results (Faust et al. 2015). For the LION study, this is particularly evident due to the Coronavirus Disease 2019 (COVID-19) pandemic, which led to several restrictions with repeated lockdowns throughout the course of the study. On that account, the participants of the LION study conducted the intervention steps under different conditions (e.g., during a lockdown or when regulations had been temporarily lifted). This entails advantages but also disadvantages that need to be mentioned. On the one hand, some participants reported having increased time

to focus on the requirements of the intervention (e.g., focusing on a healthy diet, cooking for themselves more frequently, and increasing daily physical activity). On the other hand, other participants seem to suffer from increased stress which affected compliance and motivation to keep up with the interventions. Recent findings are demonstrating that the COVID-19 pandemic has led to drastic changes in dietary practices, especially regarding cooking practices, bulk buying, and intake of different food groups and nutrients (Papakonstantinou et al. 2022), which significantly influences weight outcomes or might even have effects on postprandial metabolism. Overall, a lack of adherence is not necessarily a weakness of the study since it properly reflects behavioral challenges of daily life, that should be considered in the analysis (Gardner et al. 2020). Additionally, the controlled study environment and frequent contact between the participants and the study team further reduce comparability to a "real-life" setting, which might be amplified by the COVID-19 pandemic, since lockdowns introduced an even stricter controlled environment. In this regard, measurements of real-life conditions with wearables or other portable technologies might be of high value for future research, since humans do not live in such controlled environments.

Finally, although RCTs are of high clinical relevance and can address causality, they are often designed to reduce variation across the population. However, inter-individual variation is the main premise for personalized nutrition (Ellis et al. 2021). Therefore, findings might not be evident by design, making it difficult to determine the clinical relevance of personalized nutrition. Additionally, the evaluation of diet-induced changes in health outcomes is limited, since such changes are subtle and occur over a longer period (Lépine et al. 2022). A follow-up period of one year has been established to address this, but it is yet unclear if the duration will prove to be sufficient to accurately capture long-term weight changes in this cohort.

There are some studies investigating phenotyping as a tool to identify subgroups of people with overweight or obesity and to evaluate how different weight management strategies may work better for some individuals than others. Fiamoncini *et al.*, for example, comprehensively phenotyped 70 individuals based on their postprandial response of ~300 metabolites after a multi-nutrient meal challenge and subsequently tested how the postprandial metabolic response changes after moderate weight loss (Fiamoncini et al. 2018). Two different metabotypes were identified, and participants belonging to the "unhealthier" metabotype had higher adherence to unfavorable dietary habits and showed characteristics associated with the metabolic syndrome (e.g., impaired glucose clearance, higher visceral fat mass, elevated hepatic lipid levels) (Fiamoncini et al. 2018). Individuals of both metabotypes lost an average of 5.6 kg of weight after 12 weeks of energy restriction by 20 %, but only individuals from the "unhealthier" metabotype group significantly profited by weight loss with significant

improvements in the postprandial glycemic response. This demonstrates that phenotyping is a helpful tool to identify subgroups that respond differently to a weight loss intervention, which is not evident at a population level. However, the results of this study do not provide information if metabolic subgroups respond differently to distinct diets.

A publication based on the data acquired in the Preventing Overweight Using Novel Dietary Strategies (POUNDS) Lost study evaluated which factors determine obesity and how they can predict weight changes or improvements of other health-related metabolic outcomes after weight loss (Bray et al. 2019). POUNDS Lost is a RCT in a 2 × 2 factorial design, that included a total of 811 healthy participants aged 30 - 70 years, with a BMI between 25 – 40 kg/m², that were randomly assigned to one of four energy-reduced dietary interventions with different macronutrient compositions for 2 years (Bray et al. 2019; Sacks et al. 2009). It was revealed that genetic markers, demographic and behavioral factors, hormone status, and baseline glycemic status can predict weight loss (Bray et al. 2019; Hjorth et al. 2019b). However, this study differs from the LION study since it does not consider postprandial metabolism for phenotyping, and predicting variables were tested in isolation for their effect on weight loss without considering possible interactions.

The Diet Intervention Examining the Factors Interacting with Treatment Success (DIETFITS) trial phenotyped 609 participants with a mean age of 40 years and a mean BMI of 33 kg/m², based on their genotypes and postprandial insulin response at baseline (Gardner et al. 2018). The participants were subsequently assigned either to a healthy low fat or a healthy low carbohydrate diet aiming at weight loss, which was “matched” or “mismatched” to the identified phenotype (Gardner et al. 2018). It was assumed that a low carbohydrate diet would be superior for individuals with higher postprandial insulin secretion or greater insulin resistance since this diet reduces insulin demand possibly facilitating weight loss (Gardner et al. 2018). Although fat and carbohydrate intake was well-differentiated between intervention groups, no significant difference in weight loss was observed between the two groups (Gardner et al. 2018). Further, neither the genotype nor baseline insulin secretion was found to be associated with the dietary effects on the 12-month weight change (Gardner et al. 2018). Similarly, a systematic review equally found no evidence for genotype–diet interactions on weight loss (Bayer et al. 2020). It is interesting to note, that the dietary intervention in the DIETFITS trial had a major emphasis on diet quality. Therefore, the question arises whether the identification of phenotypes for personalized nutrition is indeed valuable, or whether only the focus on a healthy diet is enough for people at risk. Furthermore, this study focuses on weight loss as an outcome, whereas the LION study aims for long-term weight maintenance after weight loss.

It is evident that research around predictors for weight loss and weight maintenance and its determinants is still in a nascent stage and has not consistently shown clinically relevant results. Further, the effectiveness of holistic personalized approaches for long-term weight management still needs to be proven. This is especially important in light of the fact that respondents to a survey conducted in Germany consider a gene-based dietary recommendation to be an effective concept for the future (Bayer et al. 2021). There is still work to do, and lessons learned from previous studies as well as addressing remaining open questions should be considered in future research.

5.2 INTER-INDIVIDUAL VARIABILITY OF THE POSTPRANDIAL GLUCOSE RESPONSE

Clinical baseline characteristics of the LION study comply with similar cohorts in other weight loss intervention studies (Hjorth et al. 2019a; van Baak et al. 2021; Christensen et al. 2018b).

The observed PPGR shows typical trajectories, in which blood glucose levels rise shortly after glucose drink ingestion until it reaches a peak and subsequently decline after insulin secretion is stimulated (Sanders 2016; Lépine et al. 2022). In comparison with the results from the *enable* cohort, the glucose trajectories of the LION cohort resemble those of the middle-aged and older adults (Reik et al. 2022), as characteristics of these age groups are more consistent with the baseline characteristics of participants of the LION study. Similar to other studies, higher inter-individual variability of PPGR can be observed for the whole cohort in comparison to fasting glucose levels (Berry et al. 2020; Zeevi et al. 2015; Reik et al. 2022). In the *enable* cohort, variation of the PPGR was also evident between age groups (Reik et al. 2022). An age group stratification was not conducted in the LION cohort yet, however, similar variation patterns with no differences in PPGR can be observed between male and female participants. These findings are comparable to the results of the PREDICT 1 trial, which reported that sex has a small effect on glucose AUC (R^2 = 0.4) (Berry et al. 2020). In comparison, the calculated coefficient of variation of PPGR (68 %) for the PREDICT 1 cohort was higher than in the LION cohort (53 %), possibly due to the larger sample size (N = 1,002) or differences in population characteristics (e.g., mean BMI of 25.59 kg/m²) (Berry et al. 2020).

Similar to the *enable* cohort (Reik et al. 2022) clustering of the PPGR after an OGTT yielded groups with similar postprandial glucose trajectories. Two clusters were first identified, demonstrating differences in the glucose tolerance of participants. Three clusters were identified in the *enable* cohort, however, results are not entirely comparable since key characteristics of study participants (e.g., mean BMI, mean age) differ. Since people with prediabetes were also included in the LION study (Reik and Holzapfel 2020), a sub-analysis

in which clustering is carried out after stratification by glucose tolerance status might be insightful.

In total, an average of 11.8 kg (11.5 %) of body weight was lost over 8 weeks. Further changes in anthropometric measures after the weight loss intervention highlight the fact that male participants were able to lose more weight (kg and %) in comparison to females. This observation is often reported in weight loss intervention studies (Williams et al. 2015), and probably occurs due to a larger energy deficit during formula diet in males. In accordance with published results of the Prevention of Diabetes Through Lifestyle Intervention and Population Studies in Europe and Around the World (PREVIEW) study, male participants lost intra-abdominal fat to a higher extent than women (Christensen et al. 2018b). The PREVIEW study is a large multinational weight loss intervention study including participants with overweight (Christensen et al. 2018b). The weight loss intervention in this trial is similar in structure to the LION study and encompasses an 8-week formula diet as e “pre-qualifier” for further participation in long-term weight maintenance (Christensen et al. 2018b). However, this trial focuses on the prevention of diabetes onset with changes in insulin resistance being the main outcome of interest (Christensen et al. 2018b).

The baseline data assessed within the LION study provide an exhaustive set of parameters to evaluate obesity-related postprandial metabolic phenotypes. How the postprandial metabolic phenotype might predict an individual’s weight loss success after an 8-week formula-based intervention is the subject of future analyses. The application of different types of meal challenges in a crossover design and the randomized order of meal challenges for proper washout further allows the comparison of postprandial metabolism within each participant. In this context, it might also be of interest to further investigate whether the postprandial metabolic response to different meal challenges is similar, or if it is only replicable based on one type of meal challenge, as already described elsewhere (Berry et al. 2020). However, this analysis will be limited to a subgroup of the LION cohort. As described in the study protocol (Reik and Holzapfel 2020), it was originally planned to conduct two meal challenge tests at baseline. But due to delayed recruitment connected to restrictions imposed by the COVID-19 pandemic, the application of two meal challenges at baseline for each participant was repealed by an amendment of the study protocol and has come into force from November 2020. Furthermore, the interpretability of the association of postprandial metabolic phenotypes on weight loss outcomes might be skewed, since no control group was implemented in the design of the LION study. However, since individuals assigned to a control group also seem to profit from participation in an intervention study, as they also lose

weight (Bouzalmate Hajjaj et al. 2022), results comparing intervention vs. control are equally difficult to elucidate.

Overall, the results of weight loss interventions are also difficult to interpret if variation in the sex distribution of participants exists (Williams et al. 2015). The proportion of men and women in the LION study is not equal (36 % males and 64 % females), as often observed in intervention studies concerning weight management (Pagoto et al. 2012; Williams et al. 2015; Christensen et al. 2018b). Nevertheless, the LION cohort revealed a slightly higher proportion of male participants in comparison with other publications (Pagoto et al. 2012; Hjorth et al. 2019b; Christensen et al. 2018b). It is also necessary to consider, that some weight data at V2 were self-reported since some of these visits were performed as telephone-based interviews during lockdowns imposed by the COVID-19 pandemic. However, implementing telephone-based visits when an in-person visit was not possible was a worthwhile change and has allowed us to acquire a more complete dataset.

There are some studies investigating phenotypical characteristics of people to evaluate how different weight management strategies may work better for some individuals than others. Results of a publication based on data of the POUNDS Lost study suggest that baseline glycemic status can predict weight loss success (Bray et al. 2019; Hjorth et al. 2019b). However, the weight loss intervention in this trial did not include a meal-replacement formula diet, and the considered predicting variables were only measured in a fasted state (Sacks et al. 2009). The means whereby postprandial metabolism influences weight loss outcomes were elaborated in the previously mentioned work by Fiamoncini *et al.*, who identified two distinct metabotypes based on the postprandial response of several metabolites (Fiamoncini et al. 2018). After the weight loss intervention, individuals belonging to the “unhealthier” metabotype were characterized by greater metabolic improvements (Fiamoncini et al. 2018), highlighting the importance to assess postprandial metabolic phenotypes to better estimate weight loss outcomes. However, it was not within the scope of this publication to evaluate how the different metabotypes predict weight loss outcomes.

Finally, this analysis demonstrated that two clusters of postprandial glucose trajectories can be formed, providing insights into inter-individual variability of PPGR in people with obesity. The LION study includes a deep phenotyping approach that might be critical to better understand if postprandial metabolism can predict weight loss outcomes, and might be of importance for the development of personalized prevention strategies for people with obesity, following the vision to optimize weight loss strategies based on an individual’s metabolic capability of substrate utilization.

5.3 DETERMANTS OF POSTPRANDIAL GLUCOSE RESPONSE

5.3.1 HABITUAL DIET AND THE POSTPRANDIAL GLUCOSE RESPONSE

Results of the reported cross-sectional analysis assessing the effects of the habitual diet on the PPGR suggest that few of the investigated food groups and nutrients are associated with glucose AUC (Reik et al. 2022). Multiple dietary parameters, including single nutrients, food groups, or the AHEI, were considered to illustrate the habitual diet and to justify the assumed cause-and-effect relationship between diet and postprandial metabolism. Among the observed associations, the intake of carbohydrates or cereals and cereal products seem to play a role in PPGR. Overall, single findings together with the cross-sectional design of the analysis do not allow conclusions on the causality of the observed correlations. However, differences between age groups suggest that dietary factors influencing the PPGR interact with age. Therefore, no general dietary predictor for glucose AUC, that applies equally to all age groups, was identified (Reik et al. 2022).

The null association between habitual diet and PPGR is in agreement with the results stated in a recent study, that concludes that the habitual diet (estimated by a FFQ) shows an effect of < 2 % on PPGR (Berry et al. 2020). Regarding intake of isolated nutrients, the reported results contradict previous findings, where carbohydrate intake was not strongly associated with variations of PPGR (Zeevi et al. 2015; Berry et al. 2020). After clustering the study population based on the shape of their postprandial glucose trajectories, it was identified that a higher carbohydrate intake, as well as higher consumption of cereals and cereal products, indicated a greater likelihood for an individual to be assigned to a cluster characterized by a "healthier" PPGR (Reik et al. 2022). Another study involving clustering of their study population reported that a low intake of fruits and a high intake of sugar-sweetened beverages is associated with a higher diabetes risk (defined as glucose tolerance status assessed through an OGTT) in people assigned to the "unfavorable" metabotype (Riedl et al. 2020). However, it should be noted that results are not entirely comparable due to differences in methodological procedures (e.g., the definition of outcome measures, dietary assessment methods, OGTT process).

Due to the vast set of available dietary parameters in the *enable* cohort, the associations between multiple dietary exposures and glucose AUC could be examined (Reik et al. 2022). However, this also made the interpretability of the effect of diet on health-related outcomes more difficult since many possible residual confounding biases are not easily assessed and cannot be completely ruled out. For example, people do not consume nutrients or food groups in isolation. As discussed by Desmarchelier and colleagues, the food matrix might

also play a role in postprandial metabolism (Desmarchelier et al. 2019), possibly explaining why an association between the intake of cereal and cereal products and glucose AUC was found in this analysis, but none between total fiber intake alone and glucose AUC. Overall, the complexity and heterogeneity of food matrices, the diversity of dietary patterns, and interactions of food components among each other and with external factors, additionally complicate the interpretability of research published so far (Desmarchelier et al. 2019; Guo et al. 2020). The AHEI, the only evaluated parameter that most closely describes a dietary pattern as a whole, was not significantly associated with glucose AUC. Although the evaluation of dietary patterns is more generalizable than single nutrients and food groups, they are also fundamentally vague, possibly leading to varying findings (Gardner et al. 2020). Finally, the elucidation of the dietary impact on postprandial metabolism is limited due to the low accuracy of dietary assessment tools, poor representation of dietary patterns by indices, possible nutrient–nutrient interactions, and the complex classification of food quality (Asnicar et al. 2021).

The reported cross-sectional analysis provides an overview of diet-related effects on postprandial glucose metabolism (Reik et al. 2022). These findings help to better understand how the postprandial metabolism is shaped, which is of interest for the advancement of personalized nutrition approaches aiming to lower PPGR and its long-term metabolic consequences. Overall, results based on dietary assessment tools should always be interpreted with caution since such self-reported data have a great potential to introduce recall bias or misreporting. Additionally, the cross-sectional design does not allow a conclusion on the causal relationship between diet and postprandial metabolism. Therefore, it remains inconclusive how the habitual diet can influence postprandial metabolism in the long term. Large-scale intervention studies utilizing deep phenotyping of participants, such as the LION study (Reik and Holzapfel 2020), might be useful to elucidate such diet-metabolic interactions.

5.3.2 GENOTYPE AND THE POSTPRANDIAL GLUCOSE RESPONSE

The reported systematic review described a large number of studies assessing the effects of 53 different SNPs on glucose AUC, but have yielded conflicting results. Results revealed that of all the identified genes, *TCF7L2* was the most investigated gene for an association with PPGR. This is not surprising, given that this is the most significant “diabetogene” that has been extensively examined in research (Cauchi et al. 2007; Holzapfel et al. 2022). A meta-analysis, including results of nine GWAS, reported that variations within the *TCF7L2* gene are associated with postprandial 2-hour glucose concentration at genome-wide significance

(Saxena et al. 2010). The underlying mechanism involved in this process is that changes in the gene result in overexpression of transcription factor 7-like 2 protein in the pancreatic islets, causing a reduction of pancreatic insulin release and subsequently higher glucose concentrations (Lyssenko et al. 2007).

In addition, this systematic review concluded that associations observed between an evaluated SNP and glucose AUC were often reported in one single publication but were not replicated in other studies. This discrepancy was explained by several potentially confounding factors, including population characteristics, procedures of statistical analysis, or calculation basis for the outcome parameter, and reflects the lack of standardization among the studies identified in the systematic review. Further limitations complicate the comparability of the reported results. Firstly, the primary outcome of most identified articles was focused on the association between the genotype and diabetes risk. Consequently, the OGTT was used to classify the participant's glucose tolerance status. Furthermore, although the OGTT is a highly standardized meal challenge widely applied in diabetes research, discrepancies in the described OGTT procedures might also contribute to differing glucose AUCs.

The narrow focus of the research question and the explicit selection criteria for publications allowed a comprehensive and objective evaluation of the current evidence on whether the genotype might determine the overall PPGR. The observed single findings did not support robust evidence regarding an association between the genotype and PPGR. However, many determinants and their interactions can influence health outcomes, so residual confounding bias can never be completely ruled out. In that regard, the determinants that can modify the effect of the genotype on glucose AUC, such as the environment, are often difficult to accurately assess. Moreover, the estimation of the PPGR based solely on glucose AUC may provide a limited evaluation of the true impact of the genotype on postprandial glucose metabolism, since this parameter does not properly reflect the dynamic properties of the postprandial state (Erdős et al. 2021), and only represents an overall response of the glucose metabolism.

A review evaluating genetic testing for improved nutritional counseling confirmed that a single SNP has only minor effects on the overall phenotype or health outcomes (Ellis et al. 2021). High linkage disequilibrium between SNPs that are located close together similarly complicates the interpretation of the results (Grarup et al. 2014). Therefore, it has been hypothesized that polygenic scores, computed by matching several relevant SNPs, might be a better tool to predict genetic contributions to postprandial metabolism (Ellis et al. 2021). On this note, the addition of other parameters reflecting genetic variability, such as copy number

variations, short tandem repeats, and haplotypes could lead to a better understanding of the interrelationships of the genotype on postprandial glucose metabolism (Ellis et al. 2021).

In summary, although the great variability in the curriculum of the identified literature complicates interpretability, no evidence was found for an association between the reported SNPs and the investigated postprandial glucose metabolism, despite these SNPs are known to increase the risk for metabolic diseases such as diabetes. Berry *et al.* reported that a total of 32 SNPs collectively explained glucose AUC variability to a small extent (< 9 %) (Berry et al. 2020). This further supports the inquiry to what degree the genotype plays a role in postprandial metabolism and how it can translate to strategies of personalized nutrition, as has already been questioned elsewhere (Holzapfel et al. 2022).

5.3.3 Determining Factors on the Postprandial Glucose Response - Lessons Learned

Research has been mostly focused on the evaluation of one exposure at a time, showing small effects on PPGR. This step-by-step approach is crucial to obtain a better understanding of postprandial metabolism and its determinants. However, it is becoming more evident that genetic information or details on the habitual diet alone are not sufficient and precise enough to adequately characterize an individual's postprandial phenotype. Importantly, determinants and mechanisms of postprandial metabolism are highly interconnected. For example, plasma glucose is tightly regulated by insulin concentrations, which calls for the evaluation of postprandial metabolic response as a whole (Erdős et al. 2021). Further, the habitual diet was described to be associated with the diversity and composition of the gut microbiome (Asnicar et al. 2021; Christensen et al. 2018a; Guo et al. 2020). The uptake of dietary fiber, indigestible carbohydrates that enter the colon, which is subsequently fermented by gut microbiota to short-chain fatty acids (SCFAs), is one of the mechanisms involved and has regulatory effects on energy homeostasis and appetite, and might determine health outcomes (Christensen et al. 2018a; Guo et al. 2020).

The evaluated determinants of PPGR presented in this work give only small insights into the complexity of postprandial glucose metabolism. Association analysis based on linear models has limitations when analyzing such comprehensive data since separate models have to be made for each parameter, leading to non-interpretable results (Vis et al. 2015). Systemic biology approaches, incorporating genomics, proteomics, metabolomics, and microbiome analysis by principal components analysis (PCA), might be better options because such models can reduce the acquired high-dimensional data to a minimal set of interpretable parameters that are well-suited to accurately represent the complexity and variation of PPGR (Vis et al. 2015). Other outcomes representing the dynamics of the postprandial response,

such as the slope, the peak time, or the maximum and minimum concentrations, might also be needed to best characterize this type of multivariate time-series data (Vis et al. 2015; Lépine et al. 2022).

Further, early signs of metabolic inflexibility might not be optimally shown by an OGTT, because a modified PPGR is only manifested when insulin can no longer compensate for imbalances (Chia et al. 2018). Additionally, the administered glucose bolus during an OGTT does not represent a real meal and the postprandial response only exhibits the glucose metabolism, not including additional physiological systems displaying the metabolic flexibility as a whole (Stroeve et al. 2015). In this context, the use of a multi-nutrient meal challenge is probably more meaningful and better mirrors the effects of different mediators on a more complex postprandial metabolism observed in a "real-life" setting (Wopereis et al. 2017; Griffiths et al. 2020; Lépine et al. 2022). However, studies have reported that postprandial metabolic responses to a multi-nutrient meal challenge have apparent similarities to those elicited by an OGTT (Lépine et al. 2022), which speaks for the use of the OGTT as a more simple, cost-effective, and accessible method (Chen et al. 2018). However, repeated assessments of the postprandial response to a meal challenge are key for future research since they might reduce biological variability and improve the precision of estimates (Berry et al. 2020).

In conclusion, the results of the reported review and the cross-sectional analysis are observational and do not provide information on causality. Findings need to be replicated in other studies and causality needs to be tested in RCTs, by incorporating detailed phenotyping of participants and evaluating clinical endpoints (Biesiekierski et al. 2019). This highlights the importance of conducting large, appropriately powered trials with deep phenotyping tools as has been established in the LION study (Reik and Holzapfel 2020).

5.4 IMPLICATIONS FOR FUTURE RESEARCH

Earlier studies have reported greater quantifiable improvements in health outcomes using personalized nutrition approaches in comparison to generic dietary advice (Celis-Morales et al. 2017). The characterization of the postprandial metabolic phenotype for the development of personalized approaches has the potential to extend our understanding of inter-individual variation and biomarkers for metabolic flexibility, forming the bridge for the early identification of metabolic dysfunctions and an individualized evaluation of health status (Griffiths et al. 2020). However, there is not yet enough clinical evidence that can be translated into guidelines or recommendations (Bush et al. 2020). This indicates that personalized nutrition

includes, but is not limited to, the characterization of postprandial metabolic phenotypes, so there is still room for improvement.

For future developments in the field of personalized nutrition, it is important to keep in mind that with "personalization" comes some major challenges. One big hurdle is that a comprehensive assessment of the heterogeneity of an individual's metabolism is crucial to better understand which factors affect metabolic health, and how personalized nutrition can play a role in the modification of health outcomes. With that comes the need for rich and robust datasets on different characteristics of an individual (Bush et al. 2020), incorporating physiological, genetic, epigenetic, psychological, or environmental determinants (Biesiekierski et al. 2019). The assessment and evaluation of such detailed data are expensive (Celis-Morales et al. 2017), and further highlight the need for new sophisticated technologies, as well as high data quality standards (Griffiths et al. 2020).

The increasing data heterogeneity, needed for the development of personalized nutrition, might only be addressed by technological advances. Portable devices, suitable for data collection under free-living conditions already exist and are increasingly being applied in research for in-depth data collection, such as CGM or physical activity and sleep monitors (Berry et al. 2020; Holzmann and Holzapfel 2019). However, the quality and replicability of the data provided by these devices still need to be evaluated. Furthermore, high throughput technologies, incorporating machine learning and artificial intelligence, might help to manage the amount of acquired data and algorithms might even help to improve predictions. However, data generated from machine learning algorithms are very complex and possibly contentious, therefore, conclusions should be drawn with caution (Bush et al. 2020), and complex interactions between determinants should be accounted for.

Results of the Food4Me study have shown that personalized digital-based nutritional advice did improve dietary behaviors, but no differences were reported between increasing degrees of personalization (Celis-Morales et al. 2017). This raises the question of how much "personalization" is necessary to achieve optimal outcomes and if there is a "tipping point", which if exceeded, more data or information becomes uninterpretable or obsolete. Furthermore, relevant data points needed for the development of personalized nutrition strategies vary greatly, depending on the "outcome of interest" (Biesiekierski et al. 2019). Algorithms solely relying on PPGR are certainly insufficient for accurate predictions. Therefore, more basic research is needed for the identification of such next-generation biomarkers for health, that provide an optimal cost-effect balance for the desired outcome.

The analysis of such next-generation biomarkers will require holistic approaches, integrating many data points in combination with data from other disciplines, such as social, life, or computing sciences (Griffiths et al. 2020). It is still unclear, which methods for data collection, sample processing, or phenotyping approaches are optimal in this context. But it is safe to say that it will be a logistical challenge to design studies that are appropriately powered, and collect the optimal set of parameters simultaneously accounting for possible interactions (Ellis et al. 2021). Subsequently, findings need to be replicated in different populations within observational and intervention studies for the definition of robust and well-rounded clinical personalized strategies (Griffiths et al. 2020; Bush et al. 2020).

The implementation of personalized nutrition in the clinical context might also entail some hurdles. Health practitioners, for example, will likely have limited capacity to provide personalized advice based on such rich and detailed information. On that account, machine learning algorithms are also needed in the clinical context for the management and evaluation of this type of complex data, and to assist health practitioners to deliver appropriate personalized dietary advice (Bush et al. 2020). Further, increasing sampling frequencies requires a large time commitment from the individual and increases costs (Faust et al. 2015). Although sampling techniques and data management technologies are becoming more accessible, they are still not part of routine testing and day-to-day life. This can limit the applicability and accessibility of personalized services (Bush et al. 2020). Therefore, personalized nutrition might miss the mark to be widely accessible and facilitate population-wide health improvements. There is still research needed to determine if the benefits of personalized nutrition are worth the time and the price tag.

Finally, one must keep in mind that acknowledging and establishing personalized nutrition does not mean that an individual will indeed adhere to the advised lifestyle changes over the long term. Individuals have different preferences, and for this reason, some people will be better suited for less intensive alternatives (Fielding-Singh et al. 2019). Although publications are arguing that personalization might increase adherence to an intervention (Griffiths et al. 2020), it still needs more research on how and to what extent personalized nutrition can maintain or increase adherence in individuals in the long term. Nevertheless, the notion that in the future dietitians and clinicians might use the information on an individual's phenotype, along with other variables (such as blood biomarkers, genotype, and habitual diet) to provide personalized recommendations for the prevention or treatment of obesity or other diet-related diseases, seems plausible.

Notwithstanding research so far, public and private health policies continue to focus on acute-care models based on “one size fits all” approaches to treat complex and multifactorial conditions that develop over time, despite the increasing evidence that personalized nutrition can lower the cost of healthcare in the long term (Bush et al. 2020). However, the scientific committee of the European Food Safety Authority (EFSA) has recently accepted the concept of metabolic flexibility as a measure of health (Griffiths et al. 2020), going one step further in the direction of personalized nutrition. Therefore, research should focus on the identification of next-generation biomarkers reflecting metabolic flexibility and health that might be accepted by regulatory authorities in the future (Griffiths et al. 2020). This might enable changes in the healthcare system in which phenotyping based on an individual's postprandial response can be integrated into personalized prevention or treatment strategies (Ziegelstein 2017).

As mentioned previously, high throughput technologies will be key to better understanding human physiology. Digital technologies are increasingly proving to be helpful tools for data collection in clinical and daily-life settings (Berry et al. 2020; Holzmann and Holzapfel 2019). Further, digital tools are gaining interest as a novel approach to evidence-based personalized nutrition, since dietary advice can be digitally generated by algorithms based on measured or self-reported health-related data, and personal preferences and goals (Griffiths et al. 2020). Digital tools might even facilitate the interpretation of big data involved in personalized nutrition research, and improve adherence to personalized lifestyle intervention (Griffiths et al. 2020).

As an example, the ZOE app was initially designed as a supporting tool for the PREDICT 1 study, serving as a digital data collection tool for the study's participants, as well as a reminder for the tasks to be performed at specific time points during the study (Berry et al. 2020). However, based on the insights of the study, a machine learning approach was used to develop predictions of the postprandial metabolic response of an individual to different meals. In this context, ZOE became a startup and the corresponding app is now commercially available with the goal to improve health through personalized dietary advice (ZOE US Inc. 2022). A home testing kit including a blood sugar monitor and material for the collection of a stool sample is provided to the customer (ZOE US Inc. 2022). Subsequently, the data is evaluated and personalized dietary advice, with a focus on gut health and the reduction of inflammation, is administered through the app (ZOE US Inc. 2022). Similarly, DayTwo was developed based on the studies conducted at the Weizmann Institute of Science (DayTwo Inc. 2021; Zeevi et al. 2015). This program delivers personalized dietary advice to improve the glycemic response based on data on the individuals’ gut microbiome and other health-related

parameters (DayTwo Inc. 2021). Many other digital providers of personalized nutrition are emerging on the market, which are more or less evidence-based (Holzmann and Holzapfel 2019), and are often constructed of a limited number of determinants focusing on the improvement of a minimal number of outcomes. As an example, some of these companies provide an evaluation of breath samples to "reset" the metabolism, or they offer programs for weight management, digestion, or longevity based on the evaluation of blood and DNA samples.

Overall, the sheer amount of providers emerging in the field of personalized nutrition, emphasizes that the interest in personalized nutrition is increasing and in combination with digitalization, this is rising to a major trend for future developments in the health care system. This is further evident by the increasing interest of investors providing funding initiatives in this area. Recently, the National Institutes of Health (NIH) awarded a total of 170 million dollars within the Nutrition for Precision Health Fund in the U.S., for studies to develop prediction algorithms of postprandial responses to foods and the development of personalized nutrition recommendations (National Institutes of Health 2022). Similarly, the PROTEIN project has received funding from European Union's Horizon 2020 Research and Innovation program and aims to develop new technologies to provide personalized nutrition and promote healthy lifestyles in the European population (PROTEIN project 2022).

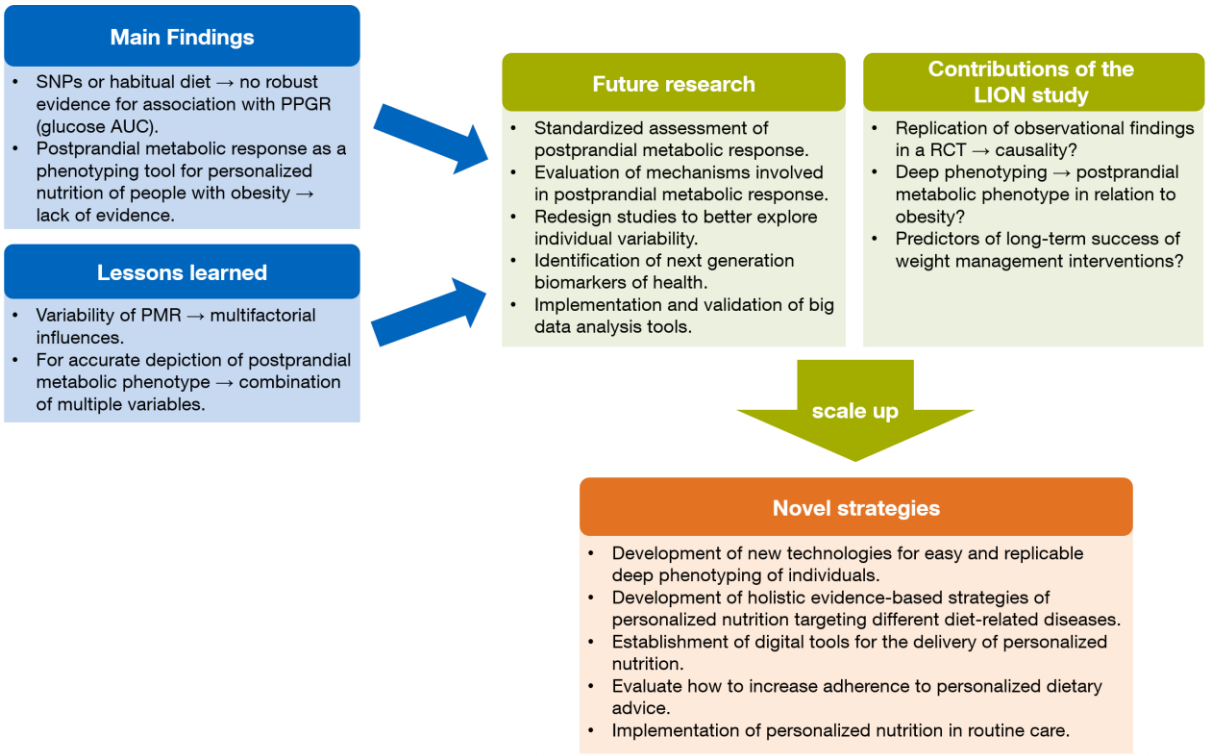

Figure 9 | From main findings and lessons learned to future research and the development of novel strategies.
Abbreviations: AUC, area under the curve; PPGR, postprandial glucose response; RCT, randomized controlled trial; SNPs, single nucleotide polymorphisms.

6 CONCLUSION AND OUTLOOK

The objective of this work was to investigate the inter-individual variability of PPGR and whether differences in the genotype and habitual diet explain variations of the PPGR after an OGTT. In addition, this work aimed to provide a better understanding of which determinants shape an individual's postprandial metabolism. Some associations of the genotype or habitual diet on the PPGR were observed; but overall, there is no robust evidence that these factors largely influence PPGR. Unreported determinants, along with unknown interactions or other "-omics" and environmental exposures might have contributed to null findings. Nevertheless, these observations suggest that single data points are not sufficient to depict the complexity of the postprandial metabolism.

Overall, the identification of postprandial metabolic phenotypes seems a promising tool to give insights into an individual's metabolic flexibility and to estimate the overall health status. In the future, if metabolic health or disease risk can be accurately measured, dietary advice can be targeted to an individual's characteristics to further optimize health in the long term. Some studies have already demonstrated that personalized nutrition is effective, showing measurable improvements in postprandial glucose metabolism and dietary behavior in the short term. However, little is known about the effectiveness of personalized nutrition in the treatment of diet-related disorders in the long term. The LION study was developed to bridge this knowledge gap. It makes use of multi-faceted data collection tools to first determine the effectiveness of two diets and two digital counseling tools on weight management. In addition, the results of this study might contribute to the identification of phenotypical differences that explain differences in weight loss and maintenance among individuals with obesity. Research in this area might provide a fundamental understanding of why treatments based on "one size fits all" approaches, that are widely established in health guidelines, have proven ineffective and have failed to successfully reduce the increasing prevalence of obesity thus far.

Some major challenges lie in future research and clinical implications of personalized nutrition. Going forward, the concept of personalized nutrition will only be made possible if simple and replicable technologies to comprehensively measure the postprandial metabolic phenotype and other individual characteristics exist. Furthermore, it is necessary to identify which set of parameters are relevant for a better resolution of an individual's metabolic flexibility. Such next-generation biomarkers of health are of great potential to identify metabolic subgroups, to which appropriate lifestyle recommendations can be made in real-time, to reshape or optimize metabolism and improve health outcomes in the long term. Reshaping dietary strategies to a more personalized approach will require wide stakeholder collaboration (e.g.,

researchers, health professionals, developers of lifestyle products, the food industry, health authorities, and public health policy advocates). However, the main aspect for this concept to work will be to encourage the individual to maintain lifestyle changes in the long term. Therefore, personalized nutrition advice should be feasible, accessible, and account for social and cultural contexts.

In conclusion, the herein presented results highlight the need for further research to get the full picture of an individual's metabolic phenotype, its determinants, and their interactions. The integration of new technologies with systematic approaches to analyze rich datasets from various disciplines will likely enable a fundamental understanding of how personalized strategies can be applied in the clinical context. The LION study will support expanding the knowledge on an individual postprandial metabolic phenotype for future evidence-based personalized dietary interventions for people with obesity. Further initiatives need to elucidate how to best implement personalized nutrition into routine care and identify possible barriers.

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APPENDIX

A1 – PROSPERO registration of the review on associations between SNPs and PPGR.

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

To enable PROSPERO to focus on COVID-19 submissions, this registration record has undergone basic automated checks for eligibility and is published exactly as submitted. PROSPERO has never provided peer review, and usual checking by the PROSPERO team does not endorse content. Therefore, automatically published records should be treated as any other PROSPERO registration. Further detail is provided here.

Citation

Sandra Bayer, Anna Reik, Hans Hauner, Christina Holzapfel. Association between genotype and the glycemic response to an oral glucose tolerance test: a systematic review. PROSPERO 2021 CRD42021231203 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021231203

Review question

Is the glycemic response to an oral glucose tolerance test dependent on the genotype?

Searches

The electronic databases PubMed, Embase, and Web of Science are systematically searched for glycemic responses to an oral glucose tolerance test in dependence on genetic variants.

The following search strategy will be used in each database:

"polymorphism" OR "genotype" OR "variant" OR "SNP" OR "genetic locus" OR "genetic loci" OR "gene locus" OR "gene loci" AND "oGTT" OR "challenge" OR "oral" OR "hour" OR "tolerance test" OR "fasting" OR "glucose tolerance" AND "glucose" OR "glycaemic" OR "glycemic" OR "postprandial" OR "response"

The search will be restricted to articles in English language, limited to human studies, and publication date since 2000. Publications in press will be included. Articles will be excluded if the study does not show data on glycemic responses to an oral glucose tolerance test in dependence on genetic variants. Furthermore, a manual search of the reference lists of all eligible articles will be supplemented.

Depending on the database, plural forms of the search terms as well as quotation marks are used. Furthermore, terms and abbreviations of search items will be searched in text word depending on the database.

Types of study to be included

Any type of study.

Condition or domain being studied

Glycemic response (e.g. glucose, area under the curve) to an oral glucose tolerance test in dependence on genetic variants

Participants/population

The review includes human studies. Studies on children and on people with specific conditions (e.g. severe diseases, pregnant or breastfeeding women, mobility impaired participants, transplant patients, people with diabetes) will be excluded.

Intervention(s), exposure(s)

The exposure to be reviewed is the number of the risk alleles of any single nucleotide polymorphism (SNP) combined with the glycemic response to an oral glucose tolerance test.

Comparator(s)/control

Risk and non-risk allele carriers of any SNP.

Context

Metabolic inflexibility has been described to have an impact on the development of obesity and diabetes type 2 (Danaher et al. 2019). The glycemic response to meals differing in the macronutrient content could be explained by genetic variants by almost 10% (Berry et al. 2020). However, the data is inconsistent. Therefore, the present systematic review will extend the current knowledge by providing an overview of studies that investigated the genetic effect on the glycemic response to an oral glucose tolerance test.

Main outcome(s)

The primary outcome of the review is the genetic effect on the glycemic response (e. g. glucose level, area under the curve) to an oral glucose tolerance test.

Measures of effect

All kinds of effect measures, e. g. relative risks, odds ratio, beta estimates, p-values.

Additional outcome(s)

None.

Measures of effect

None.

Data extraction (selection and coding)

Two independent reviewers will screen titles, abstracts, and full texts of publications according to the eligibility criteria. Any discrepancies will be discussed with a third reviewer. Studies not meeting the inclusion criteria will be excluded. Reasons for exclusion will be documented.

Data from the selected articles will be extracted independently by two reviewers to ensure compliance. Literature will be organised by Endnote. A standardised pre-piloted Excel spreadsheet will be used for data extraction of the following information:

1) Study characteristics: citation (author, year), genotype information (gene locus, SNP), statistical analyses

2) Population characteristics: study population (e.g. inclusion/exclusion criteria), sample size, nationality of participants, age, gender

3) Intervention characteristics: details of intervention, duration

4) Outcome of the study (effect size)

5) Main results and limitations of the study

Authors will be contacted to gather missing information if necessary.

Risk of bias (quality) assessment

The recommendations of the Cochrane Handbook and the PRISMA guidelines will be followed. Quality assessment of articles will be performed by two independent reviewers according to standardized tools. Discrepancies between the reviewers will be discussed and resolved by the inclusion of a third reviewer.

Strategy for data synthesis

A narrative synthesis will be provided for the findings of this systematic literature search. The review should give a wide overview of genetic effects on the glycemic response to a meal-challenge. Detailed tables of the eligible studies (e.g. study characteristics, outcomes) will be shown. If applicable, a qualitative and/or quantitative synthesis of study level statistics will be assessed.

Analysis of subgroups or subsets

This is a qualitative synthesis and it is not possible to specify subgroups in advance. Nevertheless, sensitivity analysis (e.g. age groups, body mass index groups) will be carried out if applicable.

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Type and method of review

Narrative synthesis, Systematic review

Anticipated or actual start date

18 January 2021

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31 December 2021

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Conflicts of interest

Language

English

Country

Germany

Stage of review

Review Ongoing

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

MeSH headings have not been applied to this record

Date of registration in PROSPERO

14 February 2021

Date of first submission

14 January 2021

Stage of review at time of this submission [1 change]

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	No
Risk of bias (quality) assessment	Yes	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.
The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

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