The Impact of Dietary Phosphorus on Postprandial Metabolic Responses and Markers of Metabolic Health

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ABBREVIATIONS

ACP	Amorphous calcium phosphate
ADP	Adenosine diphosphate
AI	Adequate intake
ANOVA	Analysis of variance
ApoB100	Apolipoprotein B100
ApoB28	Apolipoprotein B28
ATP	Adenosine triphosphate
ATP III	Adult Treatment Panel III
BAT	Brown adipose tissue
BCAA	Branched chain amino acids
BG	Blood glucose
BLE	Bluetooth low energy
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CaP	Pentacalcium hydroxy-triphosphate
CBT	Core body temperature
cGMP	Cyclic guanosine monophosphate
CI	Confidence interval

CKD	Chronic kidney disease
CP	Creatine phosphate
CRP	C-reactive protein
DBP	Diastolic blood pressure
DINO	Diet in nutrient out
DIT	Diet induced thermogenesis
DNA	Deoxyribonucleic acid
EE	Energy expenditure
EFSA	European Food Safety Authority
FBG	Fasting blood glucose
FDA	Food and Drug Administration
FE	Fractional excretion
FGF-23	Fibroblast growth factor 23
FGF-7	Fibroblast growth factor 7
FMD	Flow mediated dilation
GFR	Globular filtration rate
GLM	Generalized linear model
GLUT4	Glucose transporter type 4
HbA1c	Glycated hemoglobin
HDL	High density lipoprotein

HIP	Health Insurance Plan
HOMA-IR	Homeostatic model assessment for insulin resistance
iAUC	Incremental area under the curve
iCGM	Integrated continuous monitoring
IOM	Institute of Medicine
IP3	Inositol triphosphate
IQR	Interquartile range
K2	Binding capacity constant
LDL	Low density lipoprotein
MARD	Mean absolute relative difference
Max	Maximum
MEPE	Matric extracellular phosphor-glycoprotein
MetS	Metabolic syndrome
Min	Minimum
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
NaPi-2	Sodium dependent phosphate transporter
NCEP	National Cholesterol Educational Program
NDNS	National Data and Nutrition Survey
NEFA	Non-esterified fatty acids

NHANES	National Health and Nutrition Examination Survey
NPY	Neuropeptide Y
OGTT	Oral glucose tolerance test
OR	Odds ratio
OTC	Over the counter
PFC	Prospective food consumption
pH	Potential of hydrogen
PICO	Population, intervention, control, outcome
POMC	Proopiomelanocortin
PRSIMA-P	Preferred reporting items for systematic review and meta-analysis protocols
PTH	Parathyroid hormone
RDA	Recommended Dietary Allowance
REE	Resting energy expenditure
RNA	Ribonucleic acid
RNI	Reference nutrient intake
RQ	Respiratory quotient
SACn	Scientific Advisory Committee on Nutrition
SBP	Systolic blood pressure
SD	Standard deviation

SE	Standard error
SEM	Standard error of the mean
sFRP-4	Secreted frizzled-related protein 4
TEE	Total energy expenditure
TEF	Thermal effect of food
TG	Triglyceride
TRPV1	Transient receptor potential vanilloid-1
uACR	Urine albumin-creatinine ratio
UCP-1	Uncoupling protein 1
UK	United Kingdom
UL	Upper limit
US	United States
VAS	Visual Analog Scale
VKM	Norwegian Scientific Committee for Food and Environment
WC	Waist circumference

ABSTRACT

Phosphorus plays a crucial role in energy metabolism, yet its impact on postprandial metabolic responses remains insufficiently explored. This dissertation investigates the relationship between dietary phosphorus and postprandial glycemia, energy expenditure, and metabolic health through a systematic review, two controlled clinical trials, and a cross-sectional analysis of the UK National Diet and Nutrition Survey (NDNS) data.

The systematic review synthesized existing literature on phosphorus intake and metabolic outcomes, highlighting its potential effects on glucose regulation, lipidemia, and thermogenesis.

The two controlled experiments assessed the influence of dietary phosphorus on postprandial glycemic responses, diet-induced thermogenesis, and appetite regulation. Experiment 1 examined the effect of phosphorus supplementation administered alongside a refined carbohydrate test meal, while Experiment 2 investigated the impact of naturally occurring dairy phosphorus. Findings demonstrated that dietary phosphorus, in both forms, significantly modulated postprandial glucose responses and appetite-related measures. Although direct effects on core body temperature were not observed in either experiment, it is conceivable that phosphorus may have influenced thermogenesis without eliciting measurable alterations in core body temperature, especially considering its significant effect on appetite regulation.

Complementing these experimental findings, a cross-sectional analysis of the NDNS explored the associations between dietary phosphorus intake, metabolic syndrome occurrence, and its individual components. Higher phosphorus intake was linked to improved triglyceride levels, lower

diastolic blood pressure, and a reduced risk of metabolic syndrome occurrence, while a weaker direct association was observed with waist circumference.

Collectively, these findings confirm the metabolic significance of dietary phosphorus, highlighting its importance in metabolic health and providing novel insights into its role in postprandial metabolic responses. Future research should aim to further investigate the mechanistic pathways underlying the observed effects of phosphorus, while accounting for its dietary sources and bioavailability within the context of human metabolism.

Chapter 1. Introduction

1.1 Background and rational

Over recent decades, the world has been witnessing gradual but significant changes in eating behaviors, with nutritional transitions from traditional diets rich in complex carbohydrates to diets high in refined sugars, simple carbohydrates, and electrolyte-free commodities (González-Olmo et al, 2021; Popkin, 2006). Concurrently, there have been considerable global increases in physical inactivity and sedentary behavior patterns, linked to the rapid advances in technology, urbanization, and the digital era, thus leading to substantial reductions in overall energy expenditure levels (Popkin, 2006; Hallal et al, 2012). Extensive research has indicated that these overall lifestyle changes have been associated with global increases in the prevalence of metabolic disorders including insulin resistance and abdominal obesity, both classified as major public health challenges (Lin et al, 2020; Malik et al, 2013; Saklayen, 2018). A recent study of National Health and Nutrition Examination Survey (NHANES) data revealed that 40% of US adults aged 18 to 44 are insulin-resistant based on HOMA-IR measurements (Freeman et al, 2023). Likewise, the latest National Diabetes Audit showed that the incidence rate of prediabetes –also known as insulin resistance– has been steadily increasing across the years in England, surging from 3 million in 2022, to 3.6 million in 2023 – an 18% increase in only one year (NHS, 2024). The International Diabetes Federation in its turn reported that the worldwide prevalence of people living with type 2 diabetes is rising exponentially, surging from 151 million (4.6% of population) in 2000 to 463 million (9.3%) in 2019. The Federation additionally warned that if no sufficient and coordinated action was taken, the global prevalence

rate will rise to 578 million (10.2%) by 2030 and to 700 million by 2045 (Williams et al, 2020).

Similarly, the NCD Risk Factor Collaboration (NCD-RisC) published that between years 1975 and 2022, obesity rates in the world nearly tripled among women (6.6% to 18.5%), quadrupled in men (3% to 14.0%), and increased 10-fold in children aged 5 to 19 (0.9% to 9.3%), with a total of over one billion people affected by obesity, alone in 2022 (NCD-RisC, 2024). The recent NCD-RisC projections for overweight and obesity state that these trends will prospectively worsen, unless worldwide, evidence-based action is taken (NCD-RisC, 2024).

Considering these global dietary changes and health challenges, the increased consumption of energy-rich, nutrient-free foods such as sugars, refined cereals, vegetable oils, and sweeteners, has resulted in significant reductions in the intake of essential micronutrients, especially that of vitamins and minerals (Popkin, 2006; Kant, 2000). Minerals are identified as vital nutrients that serve as cofactors in various physiological and chemical processes, responsible for regulating the body's metabolism and homeostasis (Carneiro, 2013). The current research intends to shed light on the macro-mineral phosphorus, given its prominent role in energy production pathways and overall metabolism. Phosphorus is notably involved in glycolysis, gluconeogenesis, phosphorylation, and cellular insulin signaling (Bouché, 2004). It is responsible for the phosphorylation of glucose to glucose-6-phosphate, an essential step required for cellular glucose uptake and trapping, necessary for energy production (Bouché et al, 2004). In addition, phosphorus is required for the synthesis of adenosine triphosphate (ATP), the energy currency of all cells (Bonora et al, 2012). These metabolic reactions are all highly dependent on the body's

extracellular phosphorus availability, which is in turn dependent on dietary phosphorus availability (Morris et al, 1978; Solomon et al, 1990). Hence, on the metabolic level, any compromises in postprandial phosphorus levels can delay postprandial cellular uptake of glucose, hinder energy production (Kalaitzidis et al, 2005) and eventually increase the risk of developing glucose intolerance and obesity (Obeid et al, 2014).

While phosphorus intake is relatively abundant in modern diets due to the widespread use of inorganic phosphorus in food additives and synthetic sources, it is critical to distinguish between these sources and naturally occurring organic phosphorus found in dairy products, meat, and other whole foods. Inorganic phosphorus, typically added to processed foods, is highly bioavailable and rapidly absorbed by the body. However, the nutritional and metabolic effects of phosphorus from organic sources may differ substantially. The absorption and utilization of organic phosphorus are more tightly regulated by the body's physiological mechanisms, as compared to inorganic phosphorus, potentially resulting in more stable and controlled serum phosphorus levels that are conducive to optimal metabolic functioning (Calvo et al, 2014; Kawamura et al, 2018). As a result, organic phosphorus may have a more favorable impact on metabolic processes, as it is metabolized more gradually and efficiently than the rapidly absorbed inorganic phosphorus. Hence, while phosphorus intake may appear abundant from synthetic sources, it is the bioavailability and source of phosphorus that are paramount in understanding its favorable role in human metabolism.

Moreover, greater attention should be paid to phosphorus density, defined as the ratio of phosphorus intake to total energy consumption, rather than the absolute amount of phosphorus consumed. This measure more

accurately reflects the quality of the diet and its potential impact on metabolic health, as phosphorus is required not only for basic cellular functions but also for efficient energy utilization and glucose metabolism.

In light of modern dietary patterns, which are characterized by the overconsumption of convenience, energy-dense, nutrient-poor foods, there is growing concern over the insufficient intake of phosphorus from bioavailable, naturally occurring food sources. This shortfall in phosphorus intake may fail to sufficiently compensate for the heightened energy consumption, potentially leading to disruptions in metabolic processes and energy regulation. Therefore, without sufficient phosphorus intake to support metabolic processes, particularly those involved in glucose metabolism and insulin signaling, individuals may be at increased risk of developing metabolic disturbances, including insulin resistance, glucose intolerance, and obesity. This imbalance highlights the importance of not only ensuring adequate phosphorus intake but also focusing on the source and density of phosphorus within the diet to mitigate the risk of developing chronic metabolic conditions.

Remarkably, when the effect of dietary phosphorus on metabolism was investigated in animal models, it was found that phosphorus exposure resulted in lower visceral fat accumulation, higher fat oxidation rates, lower levels of non-esterified fatty acids, lower plasma insulin levels, and improved glucose tolerance, as compared to low phosphorus intake groups (Eller et al, 2011; Ellam et al, 2011; Abuduli et al, 2016). Another study in dogs demonstrated that the consumption of inorganic phosphorus-containing food led to elevated serum phosphate levels and a higher risk of vascular calcification, whereas phosphorus from organic food sources did not have the same effect (Dobenecker et al, 2021). These findings highlight

the importance of examining the role of dietary phosphorus in humans, as such research could provide valuable insights into the prevention and management of insulin resistance and obesity, given the essential role of phosphorus in glucose metabolism, diet induced thermogenesis, and overall energy metabolism.

1.2 Literature review

1.2.1 Phosphorus overview

1.2.1.1 Definition

Phosphorus is integral for sustaining life across a wide range of organisms, including humans, animals, and plants. Its nomenclature is derived from the Greek word 'phosphoros', meaning bringer of light, owing to its capacity to emit light upon exposure to oxygen (Sourkes, 1998). It is the second most abundant essential mineral in the human body after calcium, and the most abundant intracellular anion (Raina et al, 2012). Biologically, phosphorus is present in three different forms: 1) hydroxyapatite, found in bones and teeth, 2) inorganic phosphates (dihydrogen and monohydrogen phosphate), found in serum and plasma, and 3) organic phosphates, found in soft tissues and extracellular fluids (Wagner, 2024).

1.2.1.2 The phosphorus cycle

Phosphorus is an essential element for human health, and its availability is strongly influenced by human activities (Bennett et al., 2001; EPA, 2025). Phosphate rocks are mined industrially to produce fertilizers, which, when applied in agricultural practices, increase phosphorus content in crops and soils, as illustrated in **Figure 1.1**. Food processing and industrial practices often add inorganic phosphorus salts to processed and fortified foods, further contributing to human exposure. Wastewater management and industrial discharge can elevate phosphorus levels in water bodies, which in turn can contribute to dietary intake through potable water (Filippelli, 2002). However, typical concentrations of phosphorus in drinking water are generally minimal (<0.1 mg/L) and contribute only a minor fraction of total dietary intake compared with food sources (EPA, 2025).

Recognizing the pathways of the phosphorus cycle (**Figure 1.1**) provides important context for understanding phosphorus intake, absorption, metabolism, and its physiological roles, which are discussed in the following sections.

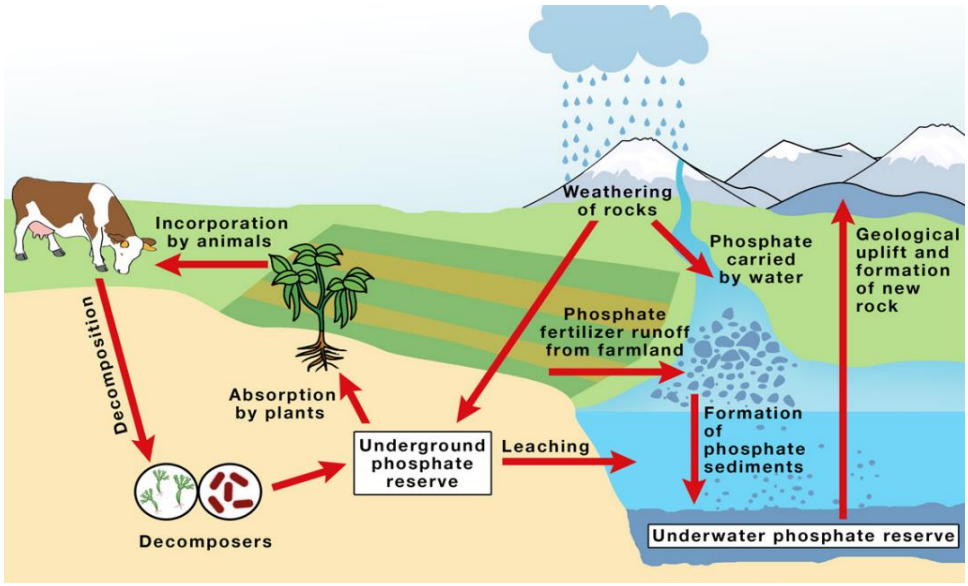

Figure 1.1. The phosphorus cycle

Schematic representation of phosphorus cycling through rocks, soils, water, sediments, and living organisms. Weathering and rainfall release phosphate from rocks into soils and water. Plants absorb phosphate, which can enter animals through consumption and become incorporated into organic molecules such as DNA. Upon decomposition, organic phosphate returns to the soil, where microorganisms mineralize it back into inorganic forms for plant uptake. Phosphorus can also be transported to rivers and oceans, where it may accumulate in sediments over time. Adapted from "Phosphorus Cycle," by S. Mukherjee, 2020, *ScienceFacts.net*. Copyright 2020 by ScienceFacts.net.

1.2.1.3 Chemistry

Phosphorus is a multivalent, non-metallic, chemical element belonging to the nitrogen family (group 15) of the periodic table of elements (Kalantar-Zadeh et al, 2010). It has an atomic symbol of *P*, an atomic number of 15, and an atomic weight of 30.97 (Kalantar-Zadeh et al, 2010). Although phosphorus has 22 isotopes, ranging from ^{26}P to ^{47}P, only one isotope is stable ^{31}P, hence classifying it as a mono-isotopic element (PubChem, 2022). Of the remaining radioactive isotopes, only two can survive long enough to be measured, ^{32}P and ^{33}P (PubChem, 2022). Phosphorus exists as a solid at room temperature. It is ranked as the 11th most abundant element in the earth's crust and is generally mined from phosphate rocks (EFSA, 2015).

Due to its high reactivity, phosphorus does not occur in nature as a free element, it is instead found in the form of inorganic phosphate minerals (PO_4^{-3}), i.e. hydroxyapatite ($Ca_{10}(OH)_2(PO_4)_6$), chlorapatite ($Ca_{10}Cl_2(PO_4)_6$) and fluorapatite ($Ca_{10}F_2(PO_4)_6$), which are abundant in rocks, soil, water and living organisms (EFSA, 2015; Sourkes, 1998). Phosphates are essential compounds composed of a phosphorus atom bound to four oxygen atoms. Their molecular structure serves as a fundamental building block for many vital biological processes and has wide-ranging applications across industries, agriculture, and modern life (Gichuhi, 2020). Phosphates occur in two main forms:

- **Inorganic phosphates,** which are free phosphate ions (PO_4^{-3}) commonly converted into phosphoric acid—a synthetic compound widely used in the food industry for pH control, preservation, and texture enhancement (Kalantar-Zadeh et al, 2010; Lampila, 2013).

- **Organic phosphates**, which are phosphate groups covalently bonded to carbon-containing biomolecules. They play essential roles in biological systems, including energy transfer, cell signaling, and structural integrity of nucleic acids.

These forms display distinct structural, functional, and solubility properties, with the main differences outlined in **Table 1.1** and their molecular structures depicted in **Figure 1.2.**

Table 1.1. Comparison between inorganic and organic phosphates

Feature	Inorganic phosphates	Organic phosphates
Chemical structure	Free phosphate ion (PO_4^{-3})	Phosphate group bound to carbon-containing molecules
Presence in nature	Found in soil, water, rocks, and bodily fluids	Found in biomolecules (e.g. phospholipids and genetic material)
Typical uses Typical uses	Fertilizers, animal feed, pesticides, asphalt, pharmaceuticals, and cosmetics	Cellular metabolism, genetic material synthesis, and cell membrane structure
Biological role	Involved in buffering systems and bone mineralization	Critical in energy transfer, genetic information, and cell membrane structure
Solubility	Generally water-soluble	Solubility depends on the attached organic molecule
Source	Derived from phosphate rocks, dietary intake, and supplementation	Synthesized within living organisms
Examples	– Phosphate (PO_4^{-3}) – Monohydrogen phosphate (HPO_4^{-2}) – Dihydrogen phosphate ($H_2PO_4^-$) – Phosphoric acid (H_3PO_4) – Ammonium phosphate ($(NH_4)_3PO_4$) – Calcium phosphate ($Ca_3(PO_4)_2$) – Sodium phosphate (Na_3PO_4)	– Adenosine triphosphate (ATP) – Deoxyribonucleic acid (DNA) – Glucose-6-phosphate – Creatine phosphate – Nicotinamide adenine dinucleotide phosphate ($NADP^+$)

Adapted from Bump, 2016 and Gichuhi, 2020.

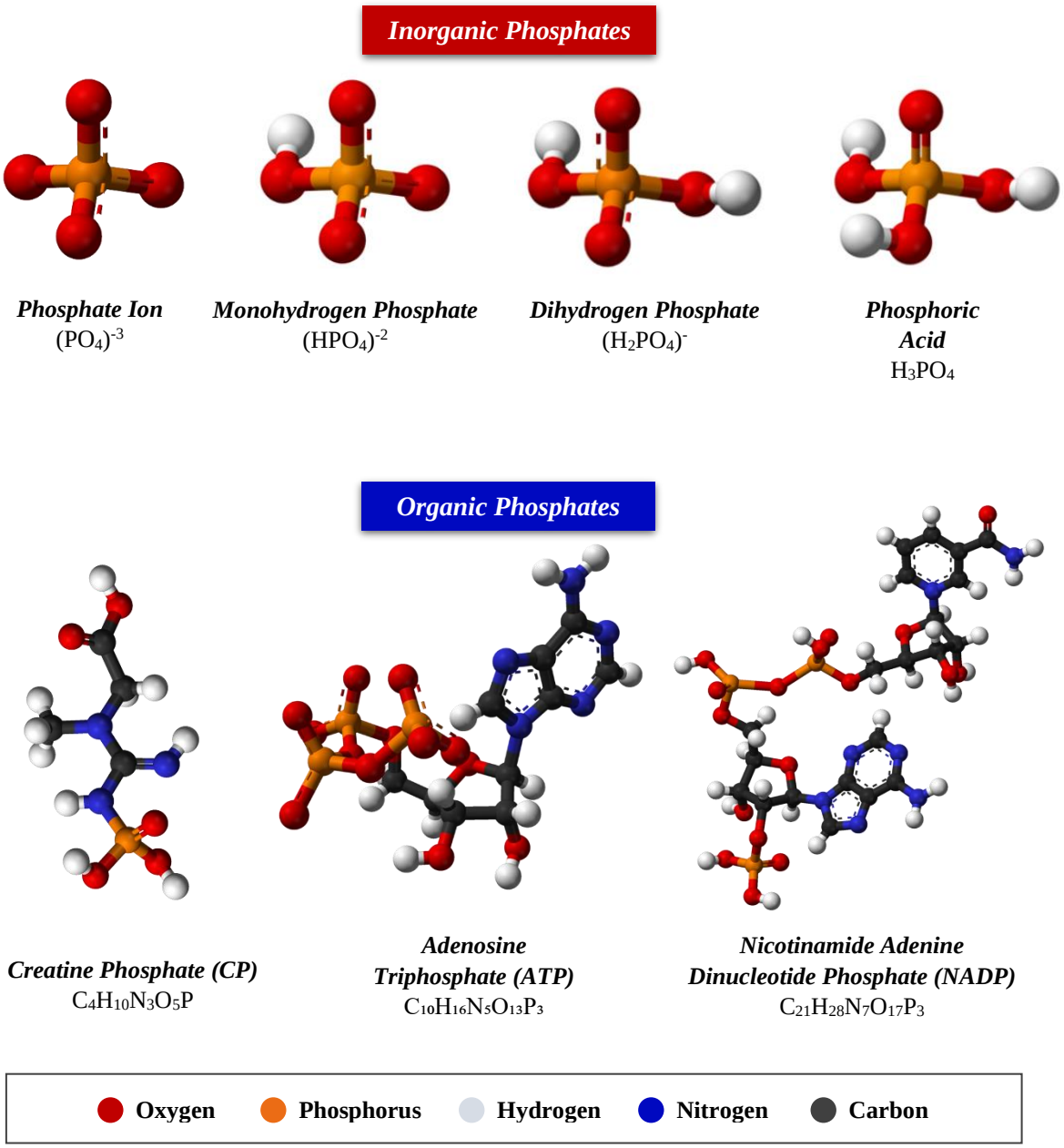

Figure 1.2. Structural differences between inorganic and organic phosphate molecules.
Adapted from Benjah-bmm27, Wikimedia Commons, https://commons.wikimedia.org/wiki/File:Phosphocreatine-3D-balls.png. Public domain.

1.2.1.4 Physiological functions

Phosphorus is an essential mineral involved in virtually every biochemical reaction taking place in the body and is pivotal for energy production. It is a component of phospholipids, which form the structural framework of cell membranes, maintaining cell integrity and regulating transport (EFSA, 2015). It is also an integral building block for the nucleic acids DNA and RNA, and thus is involved in the synthesis, storage, and transmission of genetic material (Raina et al, 2012). Moreover, phosphorus is involved in oxygen transport, by being a key component of 2,3-bisphosphoglycerate (2,3-DGP), a vital compound that mediates oxygen dissociation and release from hemoglobin in erythrocytes, ensuring its delivery to target tissues (Cade, 1984). Phosphorus is also responsible for the phosphorylation and activation processes of key enzymes, necessary for the synthesis of collagen, myelin, and other catalytic proteins, essential for overall metabolic regulation (Kalantar-Zadeh et al, 2010).

In reference to metabolism, phosphorus plays a prominent role in energy production pathways, by being a key component of adenosine triphosphate (ATP), the body's main reservoir of biochemical energy (EFSA, 2015). Other phosphorylated molecules such as creatine phosphate (CP) in muscles serve as a rapid source of phosphate for ATP production and energy transduction in substrate metabolism (EFSA, 2015). Likewise, numerous intracellular signaling processes rely on phosphorus-containing compounds such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP) and inositol polyphosphates (e.g., inositol triphosphate (IP3)) (EFSA, 2015), which are needed to ensure optimal nerve signaling and cell communication processes.

Beyond its role as a classic second messenger, phosphorus is critically involved in lipid-derived signaling molecules known as phosphoinositides (e.g., $PI(3,4,5)P_3$, $PI(3,4)P_2$, PI(3)P) (Marat, 2016). These membrane-anchored messengers regulate endocytosis, receptor recycling, and metabolic pathways by recruiting signaling proteins such as Protein Kinase B (PKB) (Marat, 2016). An overview of these pathways is shown in **Figure 1.3**, emphasizing phosphorus's central function in energy transfer, protein phosphorylation, and intracellular signaling cascades.

Phosphorus also plays essential roles in maintaining cellular and systemic homeostasis. It serves as the main intracellular buffer, thereby contributing to the regulation of acid-base balance and the prevention of disorders such as metabolic acidosis and alkalosis (Heaney, 2012). Additionally, phosphorus provides structural support to bones and teeth through its interaction with calcium to form hydroxyapatite (ESFA, 2015).

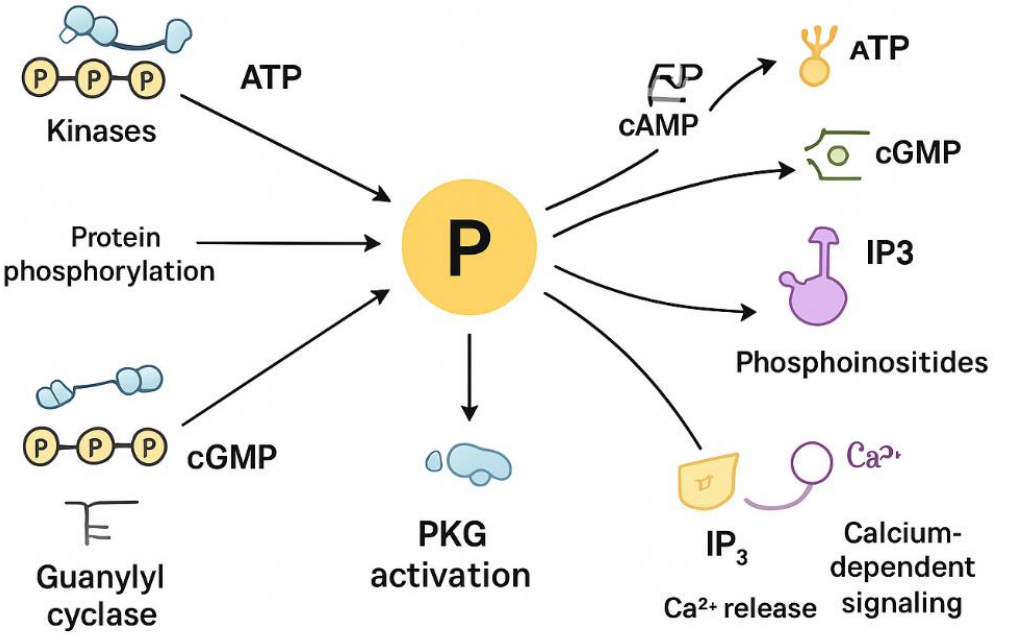

Figure 1.3. Phosphorus in cellular signaling

Schematic representation of the central role of phosphorus in cellular signaling. Phosphorus acts as a key component of adenosine triphosphate (ATP), which fuels kinase-driven phosphorylation cascades and protein activation. It also serves as a precursor for cyclic nucleotides such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which regulate protein kinase A (PKA) and protein kinase G (PKG), respectively. Inositol triphosphate (IP_3), derived from phosphoinositide cleavage, mediates intracellular calcium release and Ca^{2+}-dependent signaling. In addition, lipid-derived phosphoinositides such as $PI(3,4,5)P_3$, $PI(3,4)P_2$, and PI(3)P act as membrane-anchored second messengers, recruiting signaling proteins including Akt, and thereby regulating receptor recycling, endocytosis, and metabolic pathways. Collectively, these signaling routes demonstrate how phosphorus underpins energy transfer, enzyme activation, and intracellular communication. Adapted from Marat, 2016 and EFSA, 2015.

1.2.1.5 Dietary sources

Within the food chain, phosphorus exists in two forms: the naturally occurring organic form and the added inorganic form. Organic phosphorus is derived from animal and plant-based food sources, while the added form of phosphorus, often referred to as inorganic phosphorus salts, is commonly found in food additives, dietary supplements, enriched/fortified foods, and in processed, convenience foods. Some of the widely used phosphate additives are listed in **Table 1.2** (Tseng, 2024). Considering their versatility, phosphate salts function as flavor enhancers, emulsifiers, stabilizers, and buffers in many foods, ranging from fast foods and deli meats to canned and bottled beverages like sodas, fizzy drinks, juices and sports drinks (Kalantar-Zadeh et al, 2010; Lampila, 2013). **Table 1.3** summarizes the phosphorus content of some commonly consumed beverages (Wickham et al, 2014). Contrary to prior beliefs, carbonated beverages, especially dark colas, containing inorganic phosphate additives that were once regarded as a substantial source of phosphorus, contain considerably less phosphorus (41.3-58.3 mg/240 ml) than one glass of milk (222-247 mg/240 ml) (Karp et al, 2012; Moser et al, 2015). Other frequently overlooked sources of inorganic phosphorus are dietary supplements. Vitamin and mineral daily supplements contain an average of 108 mg of phosphorus per day (Calvo et al, 2014).

Table 1.2. Phosphate additives that are commonly used in the food industry.

E number	Phosphate additive name
E338	Phosphoric acid
E339	Sodium phosphate
E340	Potassium phosphate
E341	Calcium phosphate
E343	Magnesium phosphate
E450	Diphosphates
E451	Triphosphates
E452	Polyphosphates

Adapted from Tseng et al, 2024.

Table 1.3. Phosphorus content per serving of commonly consumed beverages.

Product	Calories per serving (kcal)	Phosphorus (mg)
Tonic Water	135	<1
Club Soda	<1	<1
Coca-Cola	145	58
Coca-Cola with Lime	146	56
Coke Zero	2	54
Diet Coke	2	27
Diet Coke with Lime	3	27
Pepsi	150	53
Diet Pepsi	<1	33
Pepsi Max	<1	42
Wild Cherry	160	53
Crush Orange	162	<1
Diet Crush Orange	<1	<1
Fanta	182	<1
Sprite	144	<1
Sprite Zero	3.6	<1
Lipton Iced Green Tea with Citrus	70	65
Mountain Dew	110	<1
Diet Mountain Dew	<1	<1
Dr. Pepper	150	54
Diet Dr. Pepper	<1	50
Dr. Pepper Cherry	150	54
Canada Dry Ginger Ale	140	<1
Aquafina Flavor Splash		
Peach Mango	<1	82
Lemon	<1	67
Wild Berry	<1	62

Adapted from Wickham et al, 2014.

Phosphorus is widely spread in food, with the main dietary contributors being animal-based foods that are rich in protein, i.e. milk and dairy products, meat, poultry, fish, and eggs; followed by nuts, baked/leavened foods, grain products, and legumes (Calvo and Uribarri, 2013). The amount of phosphorus in food is somewhat associated to protein content, though distinct differences exist. Milk and dairy products for instance, have the highest phosphorus-to-protein ratio (approximately 30 mg per gram of protein), while chicken and fish are relatively at the lower end (6 to 16 mg per gram of protein) (VKM, 2019). Food items like oils, high-fructose corn syrups, sugars and sweeteners contain negligible amounts of phosphorus (Obeid, 2013). The dietary phosphorus content of various food items is listed in **Table 1.4** (USDA, 2019).

Plant-based phosphorus (i.e., phosphorus in grains and legumes) is stored in the form of phytate. Phytate or phytic acid is a 6-fold dihydrogen ester of inositol that constitutes 60-90% of total phosphate found in plants (Kumar et al, 2010). It is known for its strong chelating properties and its ability to bind to multivalent metal ions and other minerals (Estepa et al, 1999). To get hydrolyzed, phytate necessitates the presence of the digestive enzyme phytase, which is not endogenously produced by humans, hence causing the minerals within it (including phosphorus) to be biologically unavailable (Kalantar-Zadeh et al, 2010). In addition to seeds and legumes, phytic acid resides in the germ and bran of most cereal grains, thereby positioning whole grain cereals and unleavened breads as the richest source of phytate (Harland, 1980). Yeast, on the other hand, the major ingredient of baked products, contains a phosphatase which can hydrolyze phytate to orthophosphate and inositol, thus eliminating the available binding sites, and liberating the previously chelated minerals (Harland and Harland,

1980). When the dough is incubated at warm temperatures and fermentation gets initiated, the activated phosphatase hydrolyzes phytate, thereby increasing overall inorganic phosphate and minerals' availability (Harland and Harland, 1980; Heaney, 2012). For this reason, refined cereals and cereal products, subjected to processing methods such as milling, extraction and fermentation, exhibit lower phytate levels than unrefined whole grain cereals (Hendek-Ertop et al, 2020). The phytate bound phosphorus content of selected plant-based food items are listed in **Table 1.5** (Ravindran et al, 1994).

Table 1.4. Phosphorus content of selected food items.

Food Item	P (mg/100g)	P (mg/kcal)
Milk, dairy, and eggs		
Egg, hard boiled	198	1.4
Whole milk, mozzarella cheese	412	1.3
Parmesan cheese, grated	694	1.8
Cheese, feta	337	1.3
Yogurt, plain, low-fat	137	1.9
Yogurt, plain, whole milk	95	1.6
Meats		
Chicken, breast meat, skinless, roasted	213	1.8
Beef, steak, boneless, filet	175	0.9
Turkey, meat and skin, cooked, roasted	259	1.2
Salmon, Atlantic, farmed cooked	252	1.2
Legumes and rice		
Lima beans, cooked, boiled	111	0.9
Peas, green, boiled	117	1.4
Lentils, boiled, ½ cup	179.8	1.1
Rice, brown, long grain, cooked	103	0.8
White rice, long-grain,	43	0.3
Breads, cereals and nuts		
Bread, whole wheat	212	0.8
Bread, pita, whole wheat	180	0.7
Almonds, dry roasted without added salt	481	0.8
Cashew nuts, dry roasted	593	1.1
Fruits and vegetables		
Tomatoes, ripe chopped	24	1.3
Apple	11	0.2
Clementine	21	0.4
Garlic, raw	153	1.0

Adapted from: USDA National Nutrient Database for Standard Reference Legacy of Nutrient Phosphorus, 2019.

Table 1.5. Total and phytate-bound phosphorus content of selected plant-based food items.

Food Item	Total phosphorus (g/100g)	Phytate bound phosphorus (g/100g)	Phytate-phosphorus (% of total)
Maize	0.26	0.22	84.6
Brown rice (unpolished)	0.38	0.28	73.7
Rice (polished)	0.31	0.17	54.8
Wheat Bran	1.15	1.03	76.9
Potato	0.24	0.05	20.8
Soybean	0.6	0.37	61.7
Lentils	0.31	0.2	64.5

Adapted from Ravindran et al, 1994.

1.2.1.6 Dietary intake and status

Based on data obtained from 13 dietary surveys in nine European Union countries, including the UK, mean phosphorus intake levels ranged between 1,000 and 1,767 mg/day, in adults aged 18 years and older (EFSA, 2015). In another observational study conducted across various European countries and Argentina, the median phosphorus intake was 1,388 mg/day (Su et al, 2022), with the major dietary contributors being animal-based (57.9%), followed by plant-based (35.8%) and other sources (namely processed foods) (6.3%), displaying a consistent pattern across all countries, as illustrated in **Figure 1.4.** (Su et al, 2022). Likewise, a recent Chinese cohort study reported a median dietary phosphorus intake of 933.6 mg/day among their study population (Wu et al, 2023). When analyzing the National Health and Nutrition Examination Survey (NHANES) data of the United States, the average daily phosphorus intake from both foods and supplements in years 2013-2014 was found to be 1,301 mg among women and 1,744 mg among men, as compared to 1,189 mg and 1,596 mg in years 2015-2016, respectively (USDA, 2017; USDA, 2019). Phosphate additives have been found to be significant contributors to phosphorus intake, comprising up to 30% of dietary phosphorus intake levels in the United States, owing to their high absorption rates (Calvo & Uribarri, 2013; Trautvetter et al, 2018).

Overall, concerns have been raised regarding the accuracy of assessment instruments used to estimate dietary phosphorus intake data in large-scale studies (Calvo & Uribarri, 2013; Gutiérrez, 2013), since it may not be clear whether total consumption levels are accounting for dietary phosphorus in all its forms, particularly phytate-bound phosphorus and phosphorus from inorganic sources, such as food additives and supplements (Calvo & Uribarri, 2013). The EFSA Panel, in its turn, calls for the need to identify surrogate markers of phosphorus intake, beyond dietary estimates (EFSA, 2015).

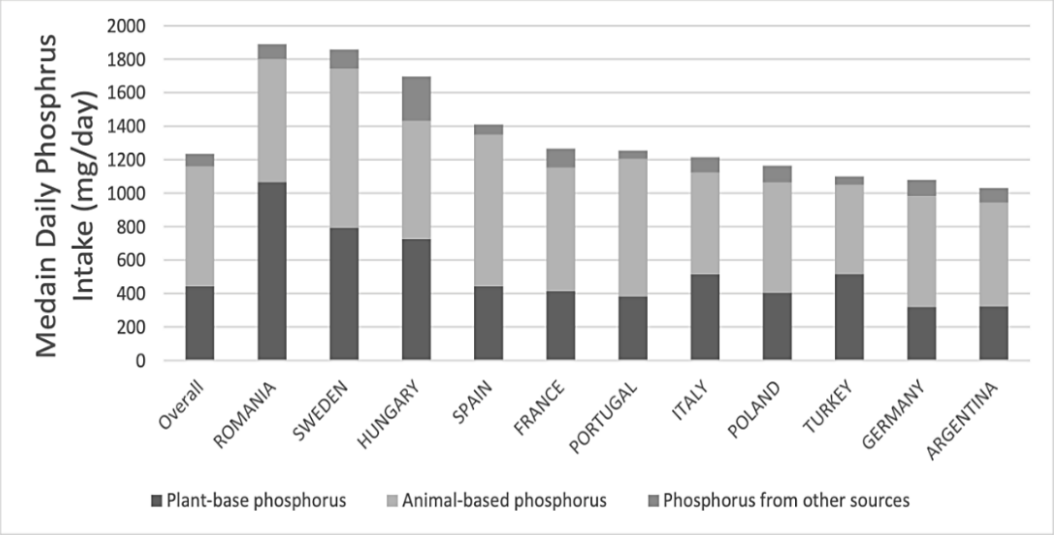

Figure 2.4. The median intake of dietary phosphorus (mg/day), stratified by food source and country.
Adapted from Su et al, 2022.

1.2.1.7 Requirements

Both, the UK guidelines, set by the Scientific Advisory Committee on Nutrition (SACN), and the European Food Safety Authority (EFSA) guidelines for the Reference Nutrient Intake (RNI) of phosphorus for healthy adults are 550 mg/day (British Journal Foundation, 2021; EFSA, 2015). The UK SACN and EFSA recommendations for phosphorus intakes among all age groups are presented in **Table 1.6**. The Panel considered that the increased RNI proposed for children and adolescents shall cover the quantity of phosphorus estimated for accretion in bone (EFSA, 2015). On the other hand, the EFSA confirmed that additional dietary phosphorus is not required for pregnant and lactating women, as long as their adequate intake, as adults, is met, which is sufficient for normal fetal growth and breast milk production (EFSA, 2015).

The American guidelines, set by the Institute of Medicine (IOM), for the recommended daily allowance (RDA) of phosphorus for adult males and females is 700 mg/day (VKM, 2017). The IOM recommendations for phosphorus intake among all age groups are presented in **Table 1.7**. Although the IOM has also set a daily tolerable upper intake level (UL) of 4 grams for adults' total phosphorus consumption, from both food and supplements (Dimeglio & Imel, 2013), yet no reports have documented any adverse effects associated with increased dietary phosphorus intake levels in humans (VKM, 2017). The IOM stated that in nearly all cases, disruption in phosphate levels in humans (i.e., hyperphosphatemia) arises from non-dietary factors, such as end-stage renal disease and vitamin D intoxication (VKM, 2017).

It is noteworthy that the EFSA Panel declares that there is no reliable biomarker of phosphorus intake or status that can be used for setting dietary reference values for phosphorus (EFSA, 2017), and thus the validity of those recommendations (both European and US guidelines) remains disputed. The current requirements were predicted based on the lower end of normal adult serum inorganic phosphorus. Correspondingly, a review on phosphorus levels and obesity reported that the RDA would have been 2100 mg/day, had it been calculated based on the median of the normal range (Obeid, 2013).

Table 1.6. The UK SACN and EFSA recommendations for phosphorus intakes (mg/day). Adequate intake (AI) for infants 7-11 months and reference nutrient intakes (RNIs) for all other groups.

Age group	UK SACN (mg/day)	EFSA (mg/day)
7-11 months	400	160
1-3 years	270	250
4-10 years	450	440
11-17 years	775	640
Adults ≥18 years	550	550
Pregnant	+ 440	550
Lactating	+ 440	550

UK, United Kingdom; SACN, Scientific Advisory Committee on Nutrition; EFSA, European Food Safety Authority.

Table 1.7. The IOM recommended daily allowance (RDA) and upper limit (UL) recommendations for phosphorus intakes (mg/day) for all age groups, both sexes.

Age group	Men (mg/day)	Women (mg/day)	UL (mg/day)
6-11 months	275	275	-
1-3 years	380	380	3000
4-8 years	500	500	3000
9-13 years	1250	1250	3000
14-18 years	1250	1250	4000
Adults >18 years	700	700	4000
Pregnant	-	700	3500
Lactating	-	700	4000

1.2.1.8 Absorption

A typical diet provides around 20 mg/kg of phosphorus per day (Raina et al, 2012). Phosphorus is usually absorbed with high efficiency, whereby absorption rate in adults ranges between 55 to 80% of customary intake, and between 65 to 90% in infants (Heaney, 2012; O'Brien et al, 2014). Intestinal absorption rate of phosphorus typically decreases with ageing (EFSA, 2015). Upon ingestion, phosphatases hydrolyze the organic forms of phosphorus to release inorganic phosphate into the gut lumen. Inorganic phosphates then get absorbed across the entire intestine, predominately in the jejunum and ileum (Sabbagh et al, 2011), via both paracellular diffusion and saturable carrier-mediated active transport system (Raina et al, 2012). Under ordinary dietary conditions, as well as with high phosphorus intake, the paracellular load-dependent passive absorption system predominates.

However, when luminal phosphorus concentrations are low, the transcellular saturable active transport system becomes the dominant mechanism (Kalantar-Zadeh et al, 2010). The transcellular active transport system necessitates the availability of sodium-dependent phosphate cotransporters NaPi-2a, NaPi-2b, and NaPi-2c, which are also expressed in the renal tubule (EFSA, 2015); with NaPi-2b being the main intestinal transporter of both phosphorus and calcium (Biber et al, 2013). Calcitriol (1,25(OH)2D), the active metabolite of vitamin D, is considered one of the main physiological determinants of intestinal phosphorus absorption, given its ability to modulate NaPi-2b transporters, hence increasing the intestinal absorption of both phosphorus and calcium (Sabbagh et al, 2011; Heaney, 2012).

1.2.1.9 Bioavailability

The amount of phosphorus absorbed is additionally dependent on several factors including the total amount of dietary phosphorus, the type of dietary phosphorus (organic versus inorganic), the food origin (animal- versus plant-derived), and the ratio of phosphorus to other minerals and dietary components (ESFA, 2015). High intakes of dietary phosphorus result in an increased phosphate absorption rate, with little evidence of an upper limit or saturation of absorption process (Wagner, 2007). Inorganic phosphorus commonly found in food additives, dietary supplements, enriched/fortified foods, some over the counter (OTC) medications and water, are more readily absorbed (around 90% absorption rate), compared to organic phosphorus naturally occurring in animal and plant-based foods; since phosphate additives are already in their inorganic, free, unbound form and do not require any enzymatic digestion (Kalantar-Zadeh et al, 2010).

Although most dietary phosphorus present in food is in the form of readily hydrolysable organic phosphate esters, yet phosphorus from animal-based food sources is more readily hydrolyzed and absorbed, compared to phytate-bound phosphorus from plant-based food sources (EFSA, 2015). Another primary factor affecting intestinal phosphorus absorption, apart from phytate, is calcium, given that both minerals are very closely related, through the actions of common regulating hormones, such as parathyroid hormone (PTH) and calcitriol (Heaney, 2012). Co-ingested calcium, in high amounts, can chelate phosphorus in the digestive chime and form insoluble complexes, thereby preventing its absorption (Heaney, 2012; O'Brien et al, 2014). Co-ingestion of high calcium to phosphorus ratio usually occurs with fortification or the use of supplements. A clinical trial that intended to examine the effect of calcium intake on phosphorus absorption, found that

every 0.5 grams increase in calcium intake, decreases phosphorus absorption by 0.166 grams, based on 470 measurements from 191 subjects (Heaney & Nordin, 2002).

1.2.1.10 Distribution

Upon absorption, phosphate enters circulation (the extracellular fluid space), and then gets trapped up by bones, teeth, and other soft tissues (Raina et al, 2012). Overall body stores of phosphorus are estimated to be around 700 grams, of which 85% is bound to calcium and localized in bones and teeth in the form of hydroxyapatite crystals (Wagner, 2007). Of the remainder, approximately 14% is found intracellularly, (of which only 5% exists in the inorganic form, and 95% exists organically in the form of ATP, ADP, CP, NAD, and 2,3-DGP), and 1% extracellularly, with an intracellular to extracellular ratio of 100:1 (Wagner, 2024; Kritmetapak, 2021). Cells maintain very small reserves of inorganic phosphorus and hence depend heavily on extracellular fluid (i.e., plasma) for a continuous supply (IOM, 1997). Phosphorus distribution in the human body is illustrated in **Figure 1.5.**

Plasma contains both organic and inorganic forms of phosphorus (Bellorin-Font et al, 2022). The organic phospholipid fraction accounts for 70% of total plasma phosphorus (Wagner, 2024). The remaining 30% of total inorganic plasma phosphorus is either bound to plasma proteins, or complexed with sodium, magnesium, or calcium, or circulates in the free ionized phosphate forms: phosphate (PO_4^{3-}), monohydrogen phosphate (HPO_4^{2-}) and dihydrogen phosphate ($H_2PO_4^-$) (Wagner, 2024) **(Figure 1.5)**. Among the free circulating forms, phosphate (PO_4^{3-}) is the most prevalent, and hence is the fragment that gets clinically measured, typically ranging between 2.5 and 4.5 mg/dl (Bellorin-Font et al, 2022). The term

'plasma phosphorus' is used in reference to the inorganic phosphorus fraction, and since inorganic plasma phosphorus is mostly in the form of the PO_4^{3-} ion, the terms 'phosphorus' and 'phosphate' are often interchangeably used in the literature (Bellorin-Font et al, 2022). Like calcium, clinically measured serum phosphate levels represent only a small fraction of total body phosphorus and hence do not accurately reflect total body stores (ESFA, 2015).

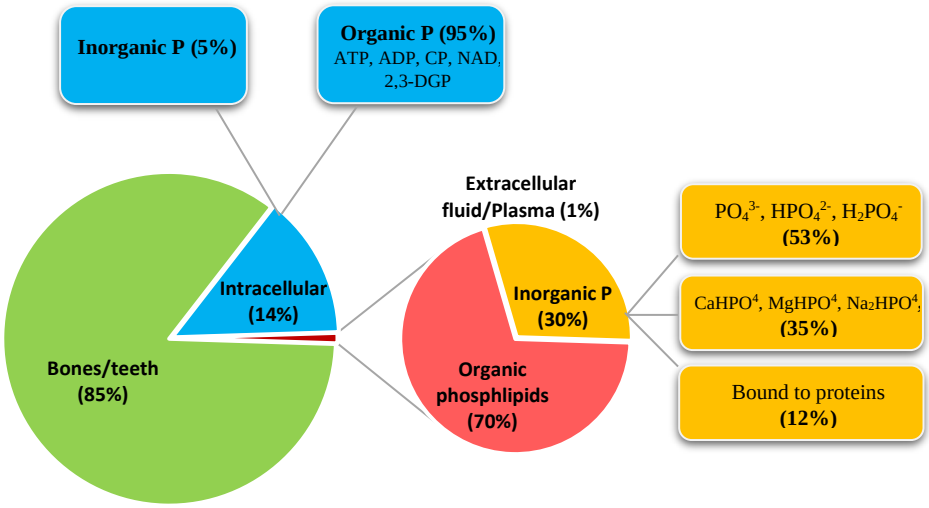

Figure 1.5. Phosphorus distribution in the human body.

P, phosphorus; ATP, adenosine triphosphate; ADP, adenosine diphosphate; CrP, creatinine phosphate; NAD, nicotinamide adenine dinucleotide 2,3-DGP, 2,3 –diphosphoglycerate; PO_4^{3-}, phosphate; HPO_4^{2-}, monohydrogen phosphate; $H_2PO_4^-$, dihydrogen phosphate; $CaHPO^4$, dicalcium phosphate; $MgHPO^4$, magnesium hydrogen phosphate; Na_2HPO^4, disodium phosphate.

1.2.1.11 Homeostasis

Normal serum phosphate concentrations are tightly controlled through homeostasis and the regulation of the freely exchanged phosphate pool. Phosphorus homeostasis refers to the process by which the body maintains a stable and balanced level of phosphorus in tissues and bloodstream. It is regulated by the bone–kidney–intestine axis through an interplay between three main physiological processes: intestinal absorption, bone remodeling (resorption and formation), and urinary and fecal losses (EFSA, 2015). The bones, kidneys, and intestines ensure that urinary losses are equivalent to phosphorus absorption, and that equal amounts of phosphorus are being deposited and resorbed from bones (Heaney, 2012; Calvo & Lamberg-Allardt, 2015). Around 3 mg/kg/day of phosphorus gets exchanged between mineralized bones and the extracellular phosphorus pool (Raina et al, 2012). Phosphate flux between body compartments is summarized in **Figure 1.6.** The primary hormones involved in regulating phosphorus homeostasis are PTH, calcitriol, and fibroblast growth factor 23 (FGF-23) (Raina et al, 2012). PTH is secreted by the parathyroid gland and is released in response to low calcium or high phosphate serum concentrations. It is responsible for increasing renal calcium reabsorption rates, by acting on the proximal tubule, eventually leading to hypocalciuria (Dimeglio & Imel, 2013). It exerts the opposite effect on phosphorus, causing declines in renal phosphorus reabsorption rates, resulting in phosphaturia. PTH correspondingly stimulates bone resorption by increasing FGF-23, calcium, and phosphate release from osteocytes (Dimeglio & Imel, 2013). It also stimulates calcitriol synthesis in the kidneys. Calcitriol is responsible for increasing both the intestinal and renal absorption of phosphorus and calcium, in addition to increasing FGF-23 and bone resorption rates, eventually leading to elevations in serum calcium and phosphorus

concentrations (Blaine et al, 2015). FGF-23 on the other hand is a key phosphatonin produced by osteocytes in the bones (Cavalli et al, 2012; Lederer, 2014). Its levels increase in response to elevations in serum phosphate. Similar to PTH, FGF-23 decreases renal phosphate reabsorption in the proximal tubule, resulting in phosphaturia (Lederer, 2014). It also decreases intestinal absorption of phosphorus by hindering calcitriol synthesis (Lederer, 2014).

An increase in serum phosphate levels (in response to a diet high in phosphorus), for example, will trigger PTH release. PTH, in its turn, activates renal phosphate excretion. The elevation in serum phosphate levels also reduces calcitriol synthesis, eventually leading to a reduced intestinal absorption rate (Berndt and Kumar, 2009; Bergwitz and Jüppner, 2010). As a response to increased serum phosphate concentrations, osteocytes conjointly accelerate their secretion of FGF-23, stimulating an increase in renal fractional excretion of phosphorus and inducing a further reduction in calcitriol concentrations, hence leading to a subsequent decrease in intestinal phosphorus absorption (Quarles, 2008). Conversely, a decline in serum phosphate levels (in response to a diet low in phosphorus) will decrease PTH release, leading to a reduced renal phosphorus excretion rate. The decrease in serum phosphorus concentrations will also result in increased calcitriol production rates, in addition to decreased FGF-23 synthesis, eventually restoring serum phosphate levels (Quarles, 2008). Recent research has shown that PTH, calcitriol, and FGF-23, are not the only regulators of inorganic phosphate homeostasis. These studies have identified that additional factors such as blood pH and phosphatonins, including secreted frizzled-related protein 4 (sFRP-4), fibroblast growth factor 7 (FGF-7) and matrix extracellular phosphor-glycoprotein (MEPE), play a vital role in maintaining phosphate balance (Shaikh et al, 2008).

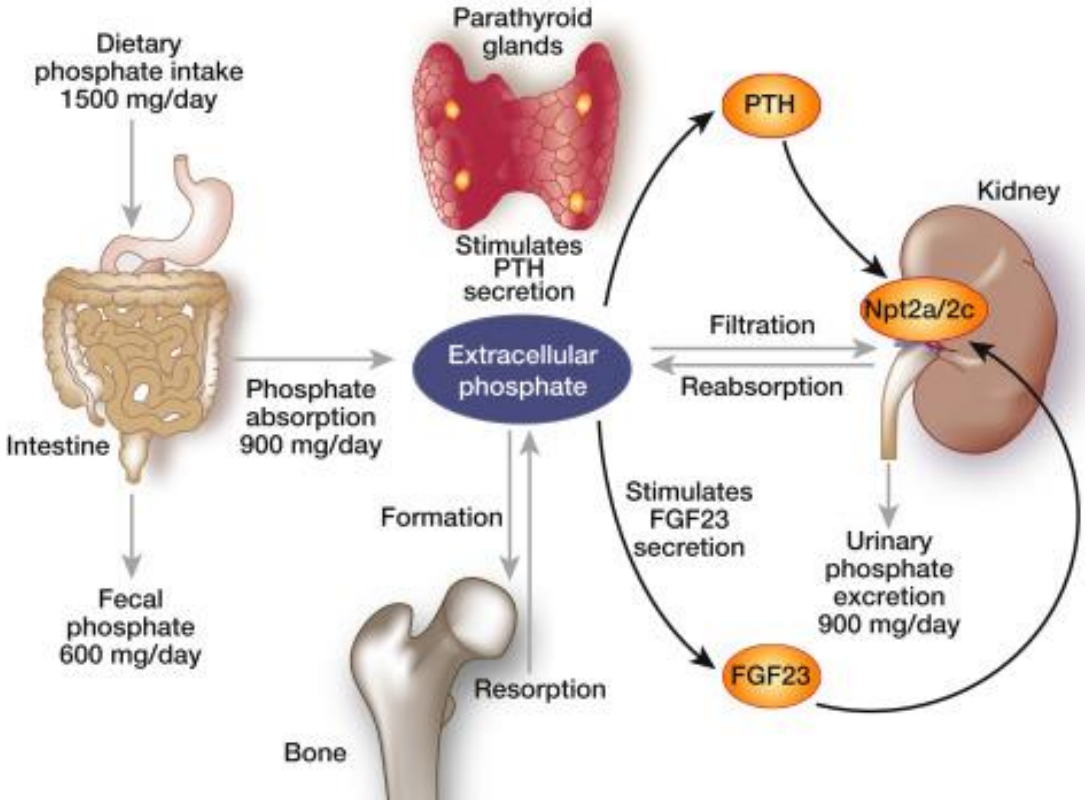

Figure 1.6. Phosphorus homeostasis in humans.
PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; Npt2a/2c, sodium dependent phosphate transporters 2a and 2c.
Adapted from Komaba & Fukagawa, 2016.

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1.2.1.12 Excretion

Renal handling of phosphorus is the utmost contributor to overall phosphate homeostasis, whereby 85% of the filtered phosphate gets reabsorbed by the proximal tubule (Dimeglio & Imel, 2013). The tubular reabsorption of phosphorus is saturable and is mediated by the transporter NaPi-2a; meaning that when serum phosphate levels surpass the renal threshold, phosphorus starts appearing in urine (Bindels et al, 2012). Normally, when the body is in a state of phosphorus equilibrium, that is, when the individual is not gaining or losing phosphorus, the amount of intestinally absorbed phosphorus is always equivalent to the amount of phosphorus excreted in urine, which is typically around 1 to 1.5 grams per day (Berndt and Kumar, 2009). The tubular reabsorption capacity adjusts to altered intakes of phosphorus within few hours, whereby urinary fractional excretion (FE) of phosphorus rises in response to a high phosphorus diet and declines with a low phosphorus diet (Bindels et al, 2012). Due to this very finely regulated renal clearance system, high dietary phosphorus intake rarely leads to major changes in serum phosphate concentrations, in people with normal kidney function (Kalantar-Zadeh et al, 2010). Factors that determine tubular phosphorus reabsorption rates are outlined in **Table 1.8.**

Fecal phosphorus excretions vary between 300 and 600 mg per day, and account for both non-absorbed phosphorus from food, and losses of endogenous phosphorus (Anderson, 2005; Delgado-Andrade et al, 2011). The latter are residues of digestive secretions (i.e. saliva, gastric juices, and bile) that have not been reabsorbed. Sweat, on the other hand, is not considered an essential route for phosphorus excretion, whereby only trace amounts of phosphorus have been detected in sweat (0.45–0.81 mg/hour) following a phosphorus-rich meal (Consolazio et al, 1963).

Table 1.8. Factors that impact tubular phosphorus reabsorption rates.

Increase P reabsorption	Decrease P reabsorption
Low dietary intake of P	High dietary intake of P
Parathyroidectomy	PTH
Hypocalcemia	Hypercalcemia
Calcitriol	Phosphatonins, i.e. FGF-23 and sFRP-4
Volume contraction	Volume expansion
Metabolic alkalosis and hypocapnia	Metabolic acidosis and hypercapnia

P, phosphorus; PTH, parathyroid hormone; FGF-23, fibroblast growth factor – 23; sFRP-4, secreted frizzled-related protein 4.

1.2.1.13 Biomarkers

Serum inorganic phosphorus concentration does not serve as a reliable biomarker for phosphorus intake levels, as it increases for only a short period of time postprandially, before it rapidly reverts to its pre-prandial, tightly regulated range (EFSA, 2015). As for phosphorus status, although serum inorganic phosphorus is a widely used clinical indicator, it does not reflect total body stores correctly. It only represents a small fraction (1%) of total body phosphorus (EFSA, 2015). To summarize, serum inorganic phosphorus concentrations generally range between 2.5 and 4.5 mg/dl in adults, irrespective of an individual's dietary phosphorus intake levels or overall body phosphorus status (Greenberg et al, 1960; IOM, 1997).

Under normal physiological conditions, urinary phosphorus concentrations typically mirror dietary intake, given that urine is the primary excretory route. Yet, urinary levels are influenced by a variety of factors that affect calcium and phosphorus metabolism, thereby limiting its use as an accurate biomarker of phosphorus intake and status (EFSA, 2015).

Both the European Food Safety Authority (EFSA) Panel and the Norwegian Scientific Committee for Food Safety (VKM) declared that to date, there are no reliable biomarkers of phosphorus intake, nor of phosphorus status, that may be used to generate validated phosphorus requirements (EFSA, 2015; VKM, 2019).

1.2.1.14 Hyperphosphatemia

Hyperphosphatemia occurs when serum inorganic phosphate concentration reaches levels greater than 5 mg/dl (Caudarella et al, 2006). Individuals with normal renal function will not develop hyperphosphatemia from an increased phosphate intake, due to the strongly regulated renal clearance capacity discussed earlier. While some studies have identified a connection between high dietary phosphorus intakes and cardiovascular, bone, and renal diseases (Gutierrez et al, 2015; Chang et al, 2014), others have found no evidence of increased health risks associated with high phosphorus intake levels (Lee et al, 2014; Chang et al, 2017; Ito et al, 2017).

Hyperphosphatemia is rather prevalent among individuals with compromised renal function, namely acute kidney injury and chronic kidney disease (CKD) (Dibartola & Willard, 2012), with a globular filtration rate (GFR) of less than 30 ml/min (stage 4 CKD) (Kestenbaum et al, 2005). It is also common in people living with hypoparathyroidism, metabolic acidosis, and erythrocyte hemolysis (Dibartola & Willard, 2012). Hyperphosphatemia eventually leads to parathyroid gland hyperplasia secondary to elevated serum PTH secretions, progressive deterioration of kidney function, renal bone disease, vascular calcification, increased risk of cardiac dysfunction, and higher mortality rates (Kuhlmann, 2006).

Severe cases of hyperphosphatemia can also result in hypocalcemia, which in turn may cause muscle cramps, arrhythmias, hypotension, and seizures (Tinawi, 2021). Treatment for chronic hyperphosphatemia involves dietary phosphate restriction (to 1000 mg/day in dialysis patients) and the use of phosphate binders, which inhibit intestinal phosphate absorption. Commonly used phosphate binders include sevelamer carbonate, calcium

carbonate, magnesium carbonate, calcium acetate, ferric citrate, sucroferric oxyhydroxide, and lanthanum carbonate (Sekercioglu et al, 2016).

1.2.1.15 Hypophosphatemia

Hypophosphatemia occurs when plasma inorganic phosphate concentration is less than 2.48 mg/dl (EFSA, 2015). It is primarily caused by metabolic disorders, and rarely from insufficient dietary phosphorus intake (EFSA, 2015). Causes of hypophosphatemia include rapid shift of phosphate from extracellular to intracellular fluid (i.e. refeeding syndrome and treatment of diabetic ketoacidosis by insulin administration), increased renal loss (i.e. hyperparathyroidism and renal tubular disorders), chronic diarrhea, starvation, vitamin D deficiency, and the excess use of phosphate binders, to name a few (Levine & Kleeman, 1994; Dibartola & Willard, 2012). Consequences of hypophosphatemia include decreased red blood cells concentrations of ATP, increased red blood cells fragility and hemolysis, platelet abnormalities, and decreased red blood cells concentrations of 2,3-DPG, which in turn result in decreased oxygen delivery to tissues (Knochel, 1977). Clinical symptoms start occurring when serum phosphorus concentrations drop below 1 mg/dl, a cut-off typically associated with total body phosphorus depletion, and include muscle weakness, fatigue, anorexia, and paralytic ileus (ESFA, 2015; Knochel, 1977). In extreme cases, reduced serum phosphate levels may impair glucose uptake by the central nervous system, resulting in metabolic encephalopathy and seizures (Levine & Kleeman, 1994). Treatment of mild hypophosphatemia involves oral replacement of phosphorus (1-2 grams per day), often administered either by milk provision (since every 1 ml of milk contains 0.9 mg of phosphorus) or by supplementation with phosphorus-containing tablets

(i.e., potassium phosphate) (Tinawi, 2021). In addition to oral replacement of phosphorus, patients with moderate to severe hypophosphatemia will require intravenous phosphorus administration, provided under close monitoring (Tinawi, 2021).

1.2.2 Phosphorus and carbohydrate metabolism

With the alarming rapid increase in type 2 diabetes prevalence around the world, there is an urgent need for extensive research aimed at its primary prevention. In this context, scientific observations have highlighted a correlation between phosphorus and glycemic control, mediated through cell glycolysis and phosphate handling in renal tubules (Wong, 2022). The mechanism of action underlying the possible link between phosphorus levels and the occurrence of hyperglycemia or diabetes is illustrated in **Figure 1.7** (Wong, 2022). Phosphorus plays an essential role in carbohydrate metabolism via phosphorylation of glucose to glucose-6-phosphate, the initial and most vital step in glycolysis, which facilitates cellular glucose uptake and intracellular glucose trapping, eventually reducing circulating blood glucose levels (Bouché et al, 2004). Inorganic phosphate is also a substrate for an important enzyme involved in glycolysis, glyceraldehyde-3-phosphate dehydrogenase, which catalyzes the conversion of D-glyceraldehyde-3-phosphate (G3P) to 1,3-diphosphoglycerate (BPG), accompanying the reduction of NAD^+ to NADH (Zhang et al, 2021).

Intake of carbohydrate-rich meals causes a rapid rise in circulating blood glucose level, as soon as intestinal glucose absorption commences. In response to elevated blood glucose concentrations, pancreatic beta cells will

proportionally secrete insulin (in healthy non-diabetic individuals). By binding to its receptors, insulin will stimulate glucose transport into the insulin-sensitive cells (skeletal muscle, adipose tissue, and the heart) via the temporary translocation of GLUT4 transporters, in addition to inhibiting hepatic gluconeogenesis, thereby clearing glucose from circulation (Chadt and Al-Hasani, 2020). Research indicates that plasma concentrations of inorganic phosphate decline immediately upon the release of insulin, as it stimulates the peripheral uptake of phosphorus (along with glucose) for the purpose of cellular substrate phosphorylation, causing a drastic decrease in postprandial serum phosphate concentrations (Pollack et al, 1934; Obeid, 2013; Ditzel & Lervang, 2010).

In view of the above, accumulating evidence revealed an inverse association between postprandial blood glucose concentrations, insulin resistance, and serum phosphate levels, independent of pancreatic insulin secretion, in healthy non-diabetic individuals (Akter et al, 2020; Backman, 2014; Friedman, 2007; Haap et al, 2006; Haglin et al, 2001; Nowicki et al, 1996). This implies that it is insulin sensitivity and not insulin secretion that is predominantly affected by low serum phosphate levels (Haap et al, 2006). The negative correlation between serum phosphate levels and HOMA-IR was also seen in children (aged 6–12 years old) living with obesity (Celik & Andiran, 2011). Khattab et al. observed that the addition of 500 mg phosphorus to the standard oral glucose tolerance test (OGTT) significantly enhanced insulin sensitivity, as evidenced by improved insulin concentrations and HOMA at 60 minutes post-ingestion, while preventing reductions in total inorganic phosphates (Khattab et al, 2005). These improvements are likely attributed to the role of phosphorus in improving insulin-mediated peripheral phosphorylation, a process highly dependent on

extracellular phosphorus availability, which in turn is dependent on exogenous phosphorus supply (Khattab et al, 2015). The need for phosphorus is therefore highest during the postprandial period, as indicated by its reduced level after glucose ingestion, and by the improvement in insulin sensitivity following its supplementation (Khattab et al, 2015).

The relation between inorganic phosphate and insulin sensitivity has been also recognized in patients diagnosed with primary hyperparathyroidism. Hypophosphatemia secondary to hyperparathyroidism was found to be significantly associated with impaired glucose tolerance and insulin insensitivity in various experimental studies (Haglin et al, 2001; Lippi et al, 2009; Haap et al, 2006; DeFronzo & Lang, 1980). Furthermore, researchers demonstrated that phosphate supplementation among insulin insensitive hypophosphatemic patients markedly enhanced insulin sensitivity and glucose tolerance scores (Wittmann & Nagy, 1997).

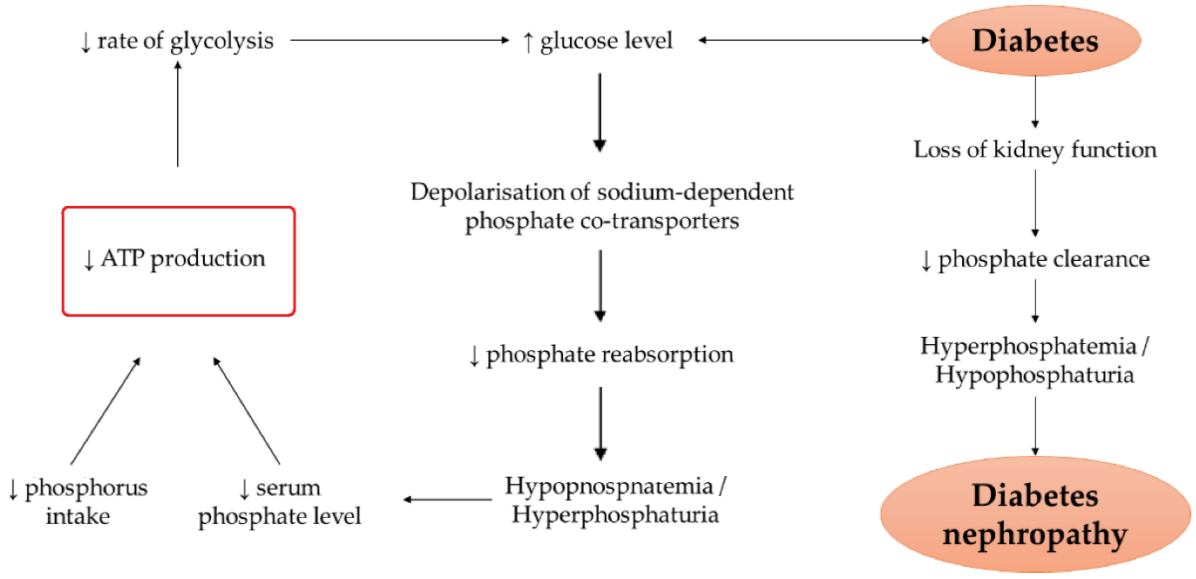


Figure 1.7. The mechanism of action underlying the possible link between phosphorus intake or circulating phosphate level and the occurrence of hyperglycemia or diabetes and its associated nephropathy.

The arrow pointing upward (↑) indicates an increase; the arrow pointing downward (↓) indicates a decrease.
Adapted from Wong, 2022.

1.2.3 Phosphorus and postprandial energy metabolism

Apart from its role in the intracellular trapping of glucose, which is a critical step in the energy production pathway, phosphorus is also an indispensable prerequisite for the synthesis of ATP, the energy currency of all cells (Bonora et al, 2012). Because ATP sustains life, and because ATP cannot be intracellularly stored, ATP availability, as represented by cellular ATP:ADP ratios, serves as the pivot point for energy balance (Tornroth-Horsefield & Neutze, 2008). The intricate relationship between ATP production and the body's metabolic responses become evident during diet induced thermogenesis (DIT). DIT, also known as thermic effect of food (TEF) or as postprandial energy expenditure (PEE), is the process by which the body expends energy to breakdown macronutrients, producing ATP, with cellular respiration in the mitochondria playing a central role (Westerterp-Plantenga et al, 1990). In other words, DIT is the increase in energy expenditure above basal resting rate that occurs after the ingestion of food components and accounts for 5%–15% of total energy expenditure (TEE), depending on type of macronutrients, micronutrients, overall body weight, and insulin sensitivity (Westerterp, 2004). Mitochondria are tasked to manufacture ATP, contributing 90-95% of total cellular energy, predominantly through oxidative phosphorylation (Nesci et al, 2021).

Although DIT is the smallest contributor to TEE, it nevertheless plays a major role in the onset and development of obesity, particularly because almost more than 50% of an average person's time is spent in the postprandial state (Obeid, 2013; Freckmann et al, 2007). DIT comprises the energy expenditure required for digestion, absorption, and metabolism of food. It is most pronounced after consuming protein-rich foods, due to the higher energy required to metabolize proteins compared to carbohydrates

or fats. Protein metabolism, in terms of protein biosynthesis and breakdown, is vastly dependent on ATP availability and supply (Pesta & Samuel, 2014). Protein biosynthesis is a very energy-intensive process, whereby 4 ATP equivalents are required for the formation of every peptide bond, or the equivalent of 0.67 kcal/1 g of protein synthesized (Hammoud et al, 2017). This process is highly reliant on phosphorus availability (Hammoud et al, 2017).

1.2.3.1 Phosphorus and diet induced thermogenesis

When the effect of dietary phosphorus on DIT was investigated in animals, studies confirmed that rats put on a high phosphorus diet for 8 weeks had increased thermogenesis compared to controls, which was manifested by increased uncoupling protein 1 (UCP-1) expression in brown adipose tissue (BAT) (Abuduli et al, 2016). In humans, the ingestion of a high-carbohydrate, low-phosphorus meal will most likely reduce phosphorus availability for ATP synthesis (in response to insulin secretion and cellular substrate uptake discussed earlier), possibly resulting in lower DIT (Obeid, 2013). This effect was demonstrated in various clinical studies, whereby phosphorus ingestion with carbohydrate meals resulted in increased DIT compared to placebo groups, further validating the association between DIT, phosphorus, and ATP availability and supply (Assaad et al, 2019; Bassil & Obeid, 2016). A series of clinical experiments have confirmed that individuals living with obesity have a blunted or diminished DIT, secondary to insulin resistance, which tends to normalize after weight loss (Segal et al, 1990; Thörne & Wahren, 1990; (Golay et al, 1982). This confirms the pivotal role of insulin resistance in thermogenesis and postprandial ATP synthesis, possibly by decreasing the peripheral cellular uptake of substrates

required for its synthesis – primarily glucose and phosphorus (Felig, 1984; Campillo et al, 1982).

1.2.3.2 Phosphorus, ATP and appetite regulation

Energy balance in humans refers to a coordinated homeostatic regulation of energy intake and energy expenditure. Maintaining a balanced energy state is essential for overall health and for obesity prevention. A substantial body of data supports the presence of physiological mechanisms that centrally regulate food intake and body weight, despite external stimuli (Watts et al, 2022). One of these regulatory mechanisms is related to postprandial ATP production and hepatic energy metabolism. Physiologically, at the central level, food intake regulation stems from signals originating from the liver through hepatic postprandial ATP metabolism (Friedman, 2007). Hepatic ATP is strongly associated with signaling satiety, through the hepatic vagal afferent activity, which detects and transmits to the central nervous system any changes in hepatic energy status, thereby influencing appetite regulation (Friedman, 2007).

An analysis of the metabolic data using the knowledge discovery in databases has revealed that decreased total and hepatic ATP levels were strongly linked to the onset and progression of obesity by signaling overeating and conserving energy (Wodek & Gonzales, 2003). In this regard, phosphorus has been found to play a significant role in appetite regulation and satiety by means of hepatic postprandial ATP production (Friedman, 2007).

Put together, the negative association between phosphorus status and food intake is mediated through the following interrelated physiological mechanisms:

1. Consumption of carbohydrate-containing foods will stimulate insulin secretion, which will in turn enhance intracellular phosphorus uptake needed for carbohydrates phosphorylation, thereby resulting in low extracellular serum phosphate levels (Brown, 2022).
2. Decreased phosphorus intake levels will limit phosphorus availability for ATP synthesis. Decreased ATP production, in its turn, will hinder thermogenesis and subsequently decrease TEE rates (Obeid, 2013).
3. The signal of low hepatic ATP synthesis will be transmitted to the central nervous system, leading to hyperphagia and increased food intake (Obeid, 2013).
4. Decreased serum phosphate levels will cause reduced 2,3-DGP levels, which will in turn lead to low oxygen availability required for oxidation, eventually resulting in decreased energy expenditure rates (Wong, 2022).

The proposed interaction between phosphorus, ATP production and obesity is illustrated in **Figure 1.8** (Wong, 2022). The overall effect of phosphorus on insulin signaling, thermogenesis, satiety and food intake further validates the reported inverse relationship found between postprandial phosphorus status and metabolic syndrome, in various observational and experimental studies (Haap et al, 2006; Kalaitzidis et al, 2005; Haglin et al, 2001).

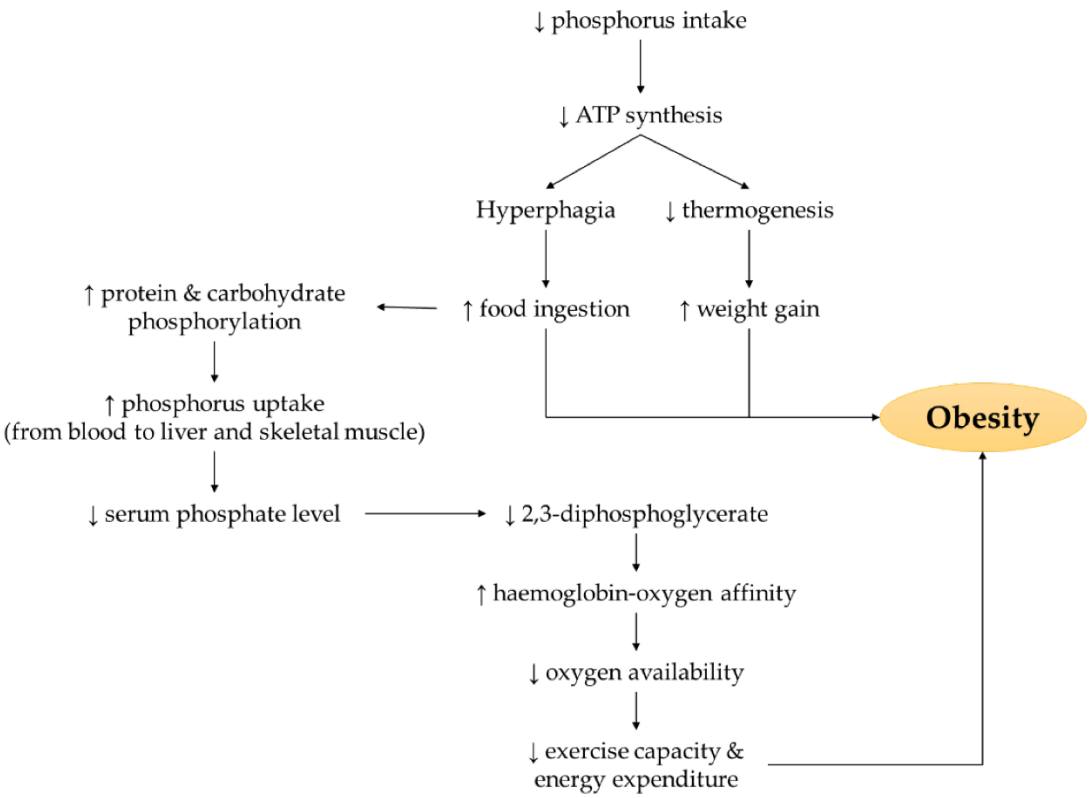


Figure 1.8. Proposed interaction between phosphorus, adenosine triphosphate (ATP) production and obesity.

The arrow pointing upward (↑) indicates an increase whereas the arrow pointing downward (↓) indicates a decrease.

Adapted from Wong, 2022.

1.2.4 Core body temperature

1.2.4.1 Overview

Core body temperature reflects the temperature of the body's internal organs, including the heart, liver, brain and blood. Thermoregulation is the process by which the body maintains its internal temperature within a stable range (close to 37 ± 0.5 °C), despite external temperature changes (Hymczak et al, 2021). Central thermoregulation relies on afferent thermal signals from core and peripheral thermoreceptors, governed by the central nervous system, primarily the hypothalamus, which acts as the body's thermostat (Hymczak et al, 2021). It is a balance between heat generated by the body (through metabolism, muscle activity/shivering, and hormonal processes) and heat lost to the environment (through sweating, vasodilation, and radiation from skin) (Hymczak et al, 2021). Body heat generation primarily results from the conversion of chemical energy in food into heat through cellular oxidative metabolism (Tansey & Johnson, 2015). ATP, essential for catalyzing cellular reactions, undergoes hydrolysis, a process where phosphate bonds are cleaved, releasing energy primarily in the form of heat (de Meis et al, 1997). Because humans are warm-blooded animals, our bodies are capable of continuously adjusting their metabolic rates in order to attain heat equilibrium (Hymczak et al, 2021). Approximately half of the daily energy expenditure in humans contribute to the maintenance of a stable CBT (Landsberg, 2012; Vinales et al, 2019). Previous studies have reported a direct relationship between energy expenditure and CBT when measured both orally and rectally (Vinales et al, 2019).

1.2.4.2 Food intake and core body temperature

The association between food intake and CBT represents a bidirectional mechanism, by which food intake influences CBT, and CBT, in turn, affects food intake. Upon ingestion of food, the body's metabolic rate temporarily increases in response to DIT, leading to a momentary rise in heat production and CBT (Westerterp, 2004). CBT plays an important role in regulating food intake through thermostatic regulatory mechanisms. The increase in metabolic heat production and CBT in response to food intake will be detected by temperature receptors in the hypothalamic region, which will in turn signal satiety and terminate eating behavior (Westerterp-Plantenga et al, 1990). CBT affects food intake at the level of pro-opiomelanocortin (POMC) neuronal activity (Jeong et al, 2018; Vicent et al, 2018). Pro-opiomelanocortin (POMC) neurons are located in the hypothalamus and are involved in regulating food intake. They typically promote the feeling of fullness and decrease food consumption, through the expression of a thermosensitive cation channel known as transient receptor potential vanilloid 1 (TRPV1) (Jeong et al, 2018).

Numerous experimental investigations have revealed a correlation between food intake and CBT. CBT increases from fasting to feeding and overfeeding, indicating that calorie intake exerts acute hyperthermic effects during feeding, while food deprivation induces hypothermia (Vinales et al, 2019; Bartfai & Conti, 2012). Studies have demonstrated that CBT decreased following 24 weeks of semi-starvation (Rising et al, 1992) and increased following a 24-hour overfeeding period with a diet composed of well-balanced proportions of macronutrients (Hoffmann et al, 2012; Reinhardt et al, 2016). Likewise, Westerterp-Plantenga et al found that normal weight individuals, who ate without restrictions, experienced a

deceleration of food intake as consumption increased, accompanied by a temperature increase in the proximity of the liver ranging from 0.8 to 1.5°C (Westerterp-Plantenga et al, 1990). This temperature elevation persisted for 60-90 minutes post-meal ingestion (Westerterp-Plantenga et al, 1990).

There are several factors that modulate CBT response to food intake at the level of the hypothalamus. During food deprivation, for instance, the concentrations of anabolic neuropeptides, including neuropeptide Y (NPY) and agouti-related protein (AgRP) increase, stimulating appetite and concomitantly reducing thermogenesis and CBT, through the sympathetic nervous system (Bartfai & Conti, 2012; Rossi et al, 1998). Signals such as glucose and insulin also influence thermoregulation by upregulating the hypothalamic expression of anorexigenic peptides, while downregulating orexigenic peptides NPY and AgRP (Bartfai & Conti, 2012). This regulatory interplay ultimately promotes an increase in CBT (Bartfai & Conti, 2012). Intriguingly, the literature remains deficient in investigating the role of phosphorus intake in this context, despite its indispensable role in carbohydrate metabolism, energy production, and appetite regulation.

1.3 Research goals, hypothesis, and significance

1.3.1 Framework and objectives

Because inorganic phosphorus is fundamental for glycolysis and ATP synthesis, and because cells store a limited amount of free inorganic phosphate, relying mostly on extracellular and exogenous sources of phosphorus to fulfill their metabolic processes, the overarching aim of this research was to investigate the independent impact of dietary phosphorus ingestion on postprandial carbohydrate metabolism and diet induced thermogenesis. To entirely serve this goal, the study was structured into three key components:

A. **Systematic review**

   A systematic review was conducted to comprehensively analyze and synthesize existing literature on the role of phosphorus in carbohydrate metabolism and energy production, identifying gaps in knowledge and informing subsequent research.

B. **Clinical trials**: to experimentally assess the effects of phosphorus on metabolic markers related to carbohydrate metabolism and energy balance through two distinct interventions – one examining the impact of exogenous phosphorus supplementation and the other evaluating the effects of phosphorus restriction.

C. **Cross-sectional study**: to evaluate real-world associations between phosphorus intake levels and metabolic parameters using data from the National Diet and Nutrition Survey (NDNS), providing insights into long-term and population-wide trends.

This multi-faceted approach aimed to integrate theoretical, experimental, and observational evidence, in order to gain a deeper, more comprehensive understanding of phosphorus and its role in improving postprandial metabolism.

1.3.2 Hypothesis

The hypothesis of this research study posits that adequate phosphorus levels will be associated with enhanced postprandial insulin sensitivity, glucose uptake, ATP production, heat generation, and overall energy balance. The opposite also holds true, whereby inadequate phosphorus levels are likely to be correlated with impaired glucose tolerance and decreased energy production, expressed as elevated postprandial circulating blood glucose levels and reduced DIT and CBT levels, respectively.

1.3.3 Significance and innovation

This research project contains several novel ideas. Given its multi-faceted structure, this study will provide a holistic view of how phosphorus influences metabolism across different study designs. It will be the first interventional study to investigate the impact of dairy-derived phosphorus on glycemia and postprandial metabolism. It will also be the first to demonstrate the distinct impact of dietary phosphorus on postprandial glycaemia and thermogenesis, in a continuous real-time mode, via the use of continuous monitoring devices for both glucose and CBT readings. Unlike single glucose readings extracted from withdrawn blood samples, a continuous glucose monitoring system provides more realistic and comprehensive insights on understanding overall glycaemia, while considering the body's various metabolic states: baseline, absorptive, and post-absorptive states.

This experiment might as well reveal the importance of phosphorus contribution in the widely known glycemic lowering ability of milk and dairy foods. Lastly, many studies focus on phosphorus supplementation, but evaluating the impact of phosphorus on metabolic responses under conditions of phosphorus restriction is an innovative approach that is rarely studied in human trials. Put together, the significance of this study lies in the advances of human nutrition in improving glucose tolerance and ultimately preventing the development of insulin resistance and type II diabetes, which has been classified as a public chronic threat.

Chapter 2. The Impact of Dietary Phosphorus on Metabolic Outcomes: A Systematic Review

2.1 Introduction

Understanding how dietary phosphorus influences postprandial metabolic responses is essential for clarifying its potential role in human health. Postprandial measures, including glycaemia, lipidemia, protein turnover, and diet-induced thermogenesis, provide insights into the body's ability to regulate nutrient handling and energy utilization following food intake. These outcomes are particularly relevant because postprandial metabolic dysfunction has been linked to increased cardiometabolic risk, even in apparently healthy adults (Dimina & Mariotti, 2019).

Evidence from human studies on the postprandial effects of dietary phosphorus remains limited and heterogeneous. Variability in study designs, participant characteristics, dietary phosphorus sources, and outcome measures has hindered the ability to draw consistent conclusions. Synthesizing the available literature through a systematic review was therefore essential for this thesis. It provided an evidence-based foundation for the subsequent experimental work by identifying relevant postprandial outcomes, guiding the design of intervention protocols, and contextualizing the expected effects of dietary phosphorus, ensuring that the experiments addressed key research gaps.

2.2 Objective

A systematic review of the literature was conducted to assess the impact of dietary phosphorus supplementation on different postprandial metabolic outcomes, namely diet-induced thermogenesis, protein turnover, postprandial glycaemia, and postprandial lipidemia, in healthy adults.

2.3 Methods

2.3.1 Protocol and guidelines

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P 2015) guidelines (Shamseer et al, 2015). Its protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO*)* with registration ID CRD42023489337.

2.3.2 Search strategy

The current review was designed to answer the following research question: what is the effect of dietary phosphorus supplementation on postprandial metabolism, as compared to the effect of low or depleted phosphorus intake levels, in healthy adults? It addressed study eligibility using the PICO (population, intervention, control and outcome) framework, presented in Table 2.1 (Howard, 2019). The search process started in June 2023 and was completed in August 2023. Electronic databases (PubMed, Google Scholar, and the Cochrane Central Register of Controlled Trials (CENTRAL)) were used to search for papers that matched the search strategy. The search strategy was designed to find all human studies that assessed the effect of dietary phosphorus on a wide range of health outcomes. Limiting the search

strategy to specific metabolic outcomes was not pertinent, since the initial searches showed very few relevant hits. Therefore, we opted to be less restrictive in the search. A detailed outline of the search strategy used for every database is provided in **Supplementary table 1.**

2.3.3 Inclusion criteria

The inclusion criteria were guided by the PICO framework (**Table 2.1**). Only experimental studies that were randomized controlled trials (RCTs), employing either parallel or crossover designs, were considered, as these intervention studies provide a robust framework for evaluating causal effects on postprandial metabolic outcomes. Observational studies, including cross-sectional and cohort designs, were excluded. Eligible participants were healthy adults aged 18–64 years, without any diagnosed diseases. Studies were included only if they assessed the primary or secondary outcomes outlined in **Table 2.1**. No restrictions were imposed regarding language, year of publication, or study setting, with eligible studies conducted in either community or hospital environments.

Table 2.1. Population–intervention–comparison–outcome (PICO) framework.

Population	Healthy male and female adults, between the ages of 18 and 64 years.
Intervention	Dietary phosphorus supplementation. Dosage: <4000 mg of phosphorus per day (which is the safe upper intake limit).
Comparison	No intervention, placebo, or phosphorus chelators.
Outcomes	Primary outcomes: postprandial metabolism, including thermogenesis (measured by substrate oxidation), postprandial glycemia (measured by postprandial glucose and insulin levels), and postprandial lipidemia (measured by postprandial lipid levels). Secondary outcomes: body composition, energy intake, and satiety (measured by appetite scores).

2.3.4 Study selection

Initial identified records were merged into one single database and duplicates were removed, using *Zotero Reference Manager*. Relevant abstracts were consequently screened using the title and abstract screening form (**Appendix A**) by a single reviewer. Full text versions were retrieved and independently reviewed by at least two authors to determine whether inclusion criteria were met.

2.3.5 Data extraction and data synthesis

A data extraction form was designed and used to recapitulate key details related to each study (**Appendix B**). Information on the author, study design, study aim, sample size, duration of the study, intervention, measured outcomes, and findings were extracted. The studies were clustered and analyzed according to three main outcome groups: 1) postprandial energy expenditure/diet-induced thermogenesis, 2) postprandial glycemia, and 3) postprandial lipidemia. A qualitative data synthesis was then performed. Due to the heterogeneity of studies, in terms of population characteristics (comparisons were made between lean and obese subjects in some studies, while not in others), type of intervention (studies administered phosphorus in different ways: with high protein meals, with carbohydrate meals, or with glucose solutions), and measured outcomes (different metabolic outcomes were measured among the studies), comparing results in a 'head-to-head' manner was not applicable, and therefore a meta-analysis could not be conducted. Additionally, most studies did not provide sufficient information on standard errors and confidence intervals and therefore could not be included in a forest plot of a meta-analysis.

2.3.6 Quality assessment

The quality of included studies was evaluated according to the Revised Cochrane Risk of Bias Tool for Randomized Trials, ROB2, 2021 (Sterne et al, 2019). Studies were assessed for the following domains: randomization process, risk of bias arising from period and carryover effects, deviations from intended interventions, missing outcome data, measurement of health outcomes, and selection of the reported result. Each domain was classified as having a high, low, or unclear risk of bias. After pooling together, an overall bias score was deduced for each study, along with a classification of it being either a high, unclear, or a low risk-of-bias study.

2.4 Results

2.4.1 Search results and study characteristics

After removal of duplicates, a total of 7061 unique abstracts were screened, 106 full-text articles were reviewed and assessed for eligibility, and 9 studies were included, as illustrated in the PRISMA 2009 flow diagram (**Figure 2.1**) (Shamseer et al, 2015). **Table 2.2** summarizes the basic characteristics of the nine included studies, which were all randomized crossover trials (n = 5 single-blinded, n = 4 double-blinded) that took place between years 2009 and 2022, and varied between 10 days to 10 months in duration. Collectively, the studies enrolled a total of 213 healthy participants, 133 males and 80 females, with their baseline body mass index ranging between normal, overweight, and obese. Phosphorus was supplemented as potassium phosphate in seven out of nine studies, and as sodium dihydrogen phosphate in two studies (Shuto et al, 2009; Volk et al, 2022). Of the nine included studies, three studies examined, as a primary aim, the effect of dietary phosphorus on energy expenditure (Abdouni et al, 2018; Assaad et al, 2018; Bassil et al, 2016); three studies tested, as a primary aim, the effect of dietary phosphorus on postprandial glycemia and lipidemia (Ayoub et al, 2015; Hazim et al, 2014; Khattab et al, 2015); one study investigated, as a primary aim, the effect of phosphorus supplementation on energy intake and appetite (Obeid et al, 2010); one study tested, as a secondary aim, the effect of phosphorus loading on postprandial glucose status (Shuto et al, 2009); and one study determined, as a secondary aim, the acute effect of dietary phosphorus supplementation on postprandial glycemia and lipidemia (Volk et al, 2022).

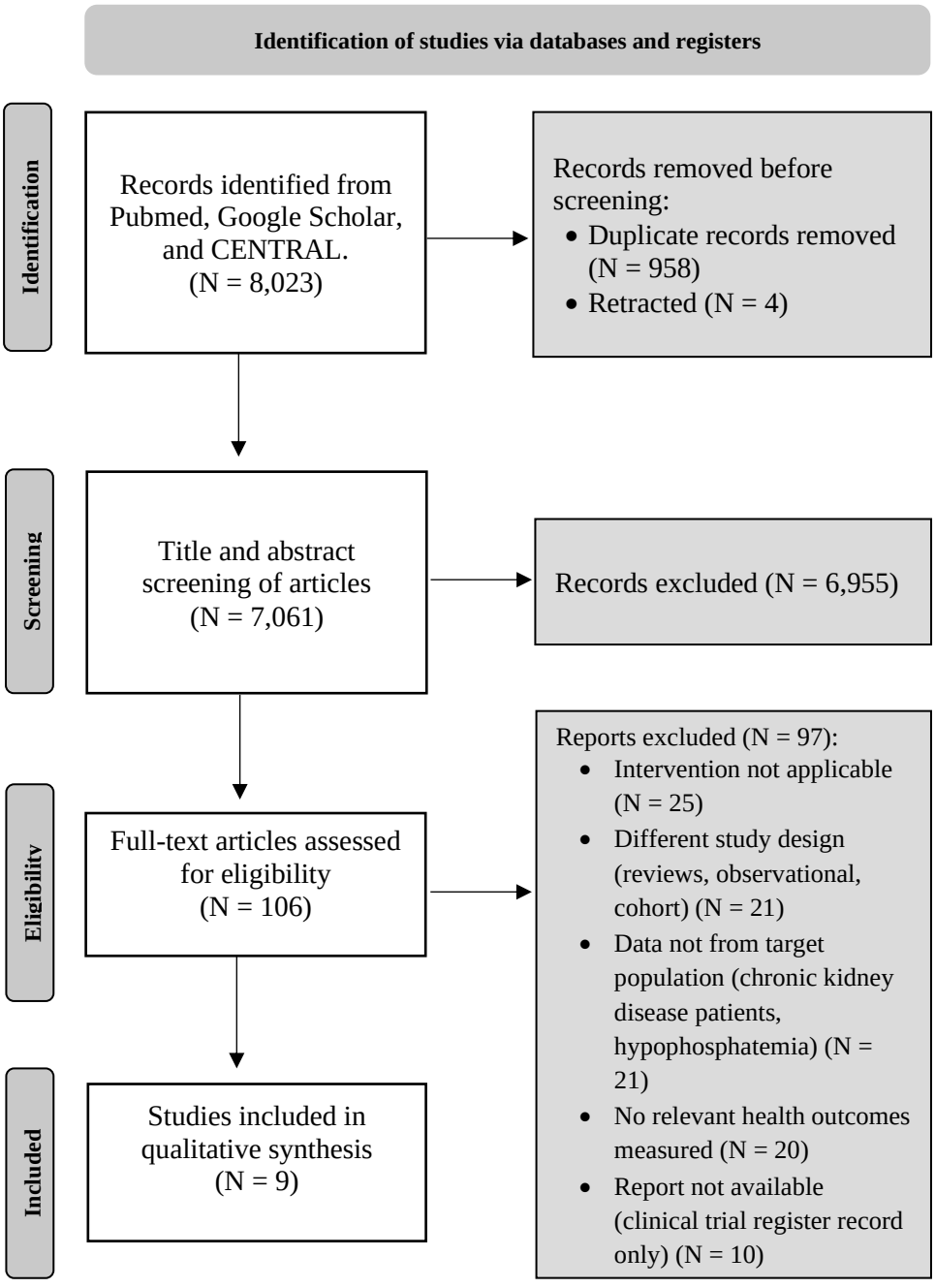


Figure 2.1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) flow diagram for included studies.

Table 2.2. Summary of the selected studies used in systematic review.

Study, year	Design	Objective	N	Population	Duration	Intervention	Measured Outcomes	Findings
Abdouni, 2018	Single-blind, randomized, crossover study	To investigate the effect of P supplementation on DIT and substrate oxidation.	12	Healthy, lean (normal BMI), male adults.	10 months (with 1-week washout period)	2 groups received isocaloric meals on 2 visits. Group1: normal protein (NPr) meal with or without P (500 mg). Group 2: high protein (HPr) meal with or without P (500 mg).	REE, RQ, substrate oxidation, urinary levels of P, creatinine, and urea nitrogen.	NPr and HPr meals had similar DIT that was increased by P ingestion (p=0.005). A significant difference was detected in DIT according to P and not to protein content of the meal.
Assaad, 2018	Single-blind, randomized, crossover study	To determine whether P supplementation with meals affects postprandial energy metabolism.	15	8 healthy, lean (normal BMI), male adults and 7 healthy, obese (BMI⩾30 kg/m2), male adults.	10 days (with 1 week washout period)	A 648 kcal meal (containing 131 mg of P) was consumed with either 4 placebo or 4 P tablets (total 500 mg).	DIT, RQ, and substrate oxidation.	P ingestion with meals increased DIT of lean and obese subjects and decreased RQ of lean subjects (p<0.01).

Table continued

Study, year	Design	Objective	N	Population	Duration	Intervention	Measured Outcomes	Findings
Bassil, 2016	Double-blind, randomized crossover study	To investigate the effect of P supplementation on DIT and satiety, in lean, overweight, and obese subjects.	23	10 healthy, lean (normal BMI), male and female adults, and 13 healthy, overweight and obese (BMI: 25-30 kg/m2), male and female adults.	10 days (with 1 week washout period)	A standard 75 g glucose solution with and without 500 mg P.	REE, DIT, RQ, substrate oxidation, and appetite scores.	P increased DIT (p<0.05) in overweight/obese subjects, a significant increase in carbohydrate oxidation rate, and decreased appetite scores (p =0.02).
Ayoub,2015	Double-blind, randomized crossover study	To examine the effects of P supplementation on body weight, BMI, WC, and subjective appetite scores in overweight and obese adults.	47	Healthy, overweight and obese (BMI⩾25 kg/m2), male and female adults.	12 weeks	Participants were requested to take with each main meal (breakfast, lunch and dinner), either three tablets of P (total 375 mg) or a placebo.	Body weight, creatinine, CRP, total cholesterol, HDL, LDL, TG, insulin, glucose, and HOMA-IR.	P decreased WC by 3.62 cm, and body weight by 0.65 kg (p <0.001). Body weight was increased by 1.13 kg in placebo group (p =0.01). Changes in appetite were reduced in P group (p <0.05). P did not affect total cholesterol, HDL, LDL, TG, glucose, insulin, and HOMA-IR levels.

Table continued

Study, year	Design	Objective	N	Population	Duration	Intervention	Measured Outcomes	Findings
Hazim,2014	Single-blind, randomized, crossover study	To determine the impact of P supplementation on insulin and postprandial lipidemia.	8	Healthy, lean (normal BMI), male adults.	10 days (with 1 week washout period)	A high fat meal (330Kcal; 69% energy from fat; 35 mg of P) with and without P (500 mg).	Serum glucose, insulin, TG, NEFA, plasma ApoB100, and ApoB28.	P increased postprandial levels of ApoB48 (p <0.05) and decreased that of ApoB100 (p <0.05), without affecting insulin, NEFA and TG levels.
Khattab,2015	Single-blind, randomized, crossover study	To investigate the effect of P supplementation on postprandial glucose and insulin status in healthy subjects.	7	<u>Experiment 1:</u> Healthy, lean (normal BMI), male adults.	10 days (with 3 days washout period)	Either 500 mg of P, 75 g glucose solution with placebo, or 75 g glucose solution with 500 mg P (G+P group).	Serum glucose, TG, insulin, and insulin sensitivity.	P significantly decreased postprandial glucose levels by 5 mg/dl. Glucose levels were lower in the G+P at 60 min (p =0.016). Insulin levels of the G+P treatment were lower than that of the glucose treatment at 60 min (p =0.002).
			8	<u>Experiment 2:</u> Healthy, lean (normal BMI), male adults.	7 days (with 3 days washout period)	Subjects were either given placebo or P (500 mg) tablets 1 hour prior to glucose ingestion.		Postprandial increase in glucose levels was similar between treatments. Glucose ingestion increased insulin levels of both treatments, but the magnitude of this increase was slightly lower in the P group.

Table continued

Study, year	Design	Objective	N	Population	Duration	Intervention	Measured Outcomes	Findings
Obeid, 2010	Single-blind, randomized, crossover study	To investigate the effect of P supplementation of a preload solution on subsequent food and energy intake.	53	Healthy, lean (normal BMI), male and female adults.	10 days (with 1 week washout period)	Either 50g of sucrose, fructose, or glucose preloads or water, with or without the addition of 500 mg of P. An ad libitum lunch was then offered.	Energy intake (ad libitum) at subsequent meal.	P addition to water preload led to 27% reduction in energy intake at subsequent meals, like that of glucose (25%). P addition to sucrose and fructose preloads led to 33% and 35% reduction in energy intake at subsequent meals, respectively.
Shuto, 2009	Double-blind, randomized crossover study	To determine the acute effect of P loading on endothelial function.	11	Healthy, lean (normal BMI), male adults.	Not reported	Subjects were alternately served meals containing 400 mg or 1200 mg of P.	Blood pressure, FMD, serum glucose, TG, HDL, and LDL.	Serum glucose levels at 2 hours post-ingestion were not significantly different between groups. FMD correlated inversely with serum P.
Volk, 2022	Double-blind, placebo-controlled crossover study	To investigate the acute effect of excessive dietary P administered on postprandial levels of serum phosphate and FGF23.	29	Healthy male and female adults; (BMI between 18.5 - 29.9 kg/m2).	8 weeks (with 4 to 7 weeks washout period)	Either 700 mg of P or a sodium-adjusted placebo in combination with a test meal.	Plasma P levels, PTH, FGF-23, glucose, insulin, TG, cholesterol (total, HDL, LDL), and ApoB48.	P increased plasma P level ($P<0.001$) and stimulated the release of PTH ($p<0.001$). FGF23 levels did not change. Postprandial glucose, insulin, and lipids levels were not affected by P vs placebo.

P, phosphorus; DIT, diet-induced thermogenesis; BMI, body mass index; REE, resting energy expenditure; RQ, respiratory quotient; WC, waist circumference; CRP, C-reactive protein; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TG, triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance; NEFA, non-esterified fatty acids; ApoB100, apolipoprotein B100; ApoB28, apolipoprotein B28; FMD, flow-mediated dilation; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; ApoB48, apolipoprotein B48.

2.4.2 Phosphorus and thermogenesis

All three studies assessing the impact of dietary phosphorus on energy expenditure reported a significant positive association with diet-induced thermogenesis (DIT) **(Table 2.2)**. Abdouni et al. (2018) compared meals of varying protein content (15% vs. 30% energy from protein; ~750 kcal) with or without 500 mg phosphorus. Energy expenditure, respiratory quotient (RQ), and substrate oxidation were measured every 30 minutes for 4 hours. Phosphorus supplementation significantly increased diet-induced thermogenesis (DIT) by ~16% across both protein conditions ($p = 0.005$), independent of protein level. No significant effects were seen on RQ or substrate partitioning.

Assaad et al. (2018) examined the effect of adding 500 mg phosphorus to a mixed meal (648 kcal; 56% carbohydrate, 30% fat, 14% protein) in lean and obese adults. Energy expenditure and RQ were measured over 240 minutes. Phosphorus significantly increased postprandial energy expenditure by ~20% relative to placebo ($p < 0.001$). In lean participants, phosphorus also reduced RQ after 120 minutes (from 0.91 ± 0.01 to 0.88 ± 0.01, $p < 0.01$), suggesting enhanced fat oxidation. No significant RQ changes were observed in obese participants.

Bassil et al. (2016) tested the effect of 500 mg phosphorus co-ingested with 75 g glucose in overweight/obese adults. Energy expenditure, carbohydrate oxidation, and appetite ratings were measured at fasting and at 30-minute intervals for 3 hours. Phosphorus increased DIT by 23% compared with glucose alone ($p < 0.05$), reflected in a higher cumulative area under the curve for postprandial energy expenditure. Carbohydrate oxidation was significantly elevated at 60 minutes post-ingestion, and participants

reported reduced appetite ratings following phosphorus supplementation (discussed in detail in **Section 2.3.5)**.

Collectively, these findings demonstrate that acute phosphorus supplementation enhances postprandial thermogenesis, accompanied by shifts in substrate oxidation, particularly towards increased carbohydrate and fat utilization.

2.4.3 Phosphorus and postprandial lipidemia

Three clinical studies have investigated the role of phosphorus in postprandial lipid metabolism, with differing protocols and outcome measures **(Table 2.2)**. Hazim et al. (2014) conducted an acute crossover trial in healthy men, where participants consumed a high-fat meal (330 kcal, 69% of energy from fat) either alone or with 500 mg of supplemental phosphorus. Blood samples were collected hourly for 6 hours. Phosphorus supplementation did not alter circulating triglycerides, insulin, or non-esterified fatty acids compared to the control condition. However, it produced significant apolipoprotein shifts, with plasma ApoB48 increasing by approximately 17% and ApoB100 decreasing by approximately 11% relative to the control meal (both $p < 0.05$).

In contrast, Ayoub et al. (2015) evaluated the chronic effects of phosphorus supplementation in overweight and obese adults. Participants were randomized to receive either phosphorus (375 mg as calcium phosphate with each main meal, totaling ~1,125 mg/day in addition to diet) or placebo for 12 weeks. Fasting and postprandial lipid parameters were assessed, including total cholesterol, HDL, LDL, and triglycerides. After the 12-week

intervention, no significant differences were observed between groups in any lipid marker.

More recently, Volk et al. (2022) investigated the acute effect of a higher phosphorus load (700 mg sodium dihydrogen phosphate) co-ingested with a mixed meal (~800 kcal; 50% carbohydrate, 35% fat, 15% protein) in healthy adults using a crossover design. Lipid and lipoprotein markers were measured at baseline and hourly for up to 6 hours. Similar to Ayoub et al. (2015), phosphorus supplementation did not significantly affect postprandial triglycerides, total cholesterol, LDL, HDL, or ApoB100 compared to placebo. ApoB48 levels were also not substantially different between conditions, with only a modest, non-consistent reduction observed at the 4-hour time point (~9%, $p = 0.04$), which was not sustained across the full postprandial period. Collectively, these findings show that while Hazim et al. (2014) identified acute changes in ApoB48 and ApoB100 following phosphorus co-ingestion with fat, these effects have not been consistently replicated in either chronic supplementation (Ayoub et al, 2015) or acute mixed-meal challenges with higher phosphorus doses (Volk et al, 2022).

2.4.4 Phosphorus and postprandial glycemia

Five studies evaluated the impact of dietary phosphorus on postprandial glycemia and insulin responses, including cases where glycemia was assessed as a secondary outcome **(Table 2.2)**. Ayoub et al. (2015) investigated chronic phosphorus supplementation (375 mg per meal; ~1,125 mg/day) for 12 weeks in overweight and obese adults and reported no significant differences in fasting or postprandial glucose, insulin, or HOMA-IR between phosphorus and placebo groups. Postprandial samples

were collected at baseline and 120 minutes, but no significant treatment effect was observed.

Hazim et al. (2014) examined the acute effect of 500 mg phosphorus co-ingested with a high-fat test meal (330 kcal; 69% energy from fat). Glucose and insulin were measured every 30 minutes over 4 hours, and no significant differences were found between phosphorus and control conditions, with mean glucose excursion remaining within ±5% of baseline in both groups.

Shuto et al. (2009) tested meals containing either 400 mg or 1,200 mg phosphorus in healthy males and assessed postprandial glucose at a single 2-hour time point. No significant difference in serum glucose was observed between treatments, with mean levels remaining ~95–100 mg/dL in both conditions.

Volk et al. (2022) evaluated acute ingestion of 700 mg phosphorus versus placebo alongside a standardized meal, with glucose and insulin measured up to 6 hours postprandially. Results showed no significant effect of phosphorus on postprandial glucose or insulin, with peak glucose levels (~110–115 mg/dL) and insulin responses (~40–50 µU/mL) comparable between conditions.

Khattab et al. (2015) reported contrasting results. In experiment 1, participants consumed a 75 g glucose solution either alone or with 500 mg phosphorus, and glucose and insulin were measured every 30 minutes for 2 hours. Co-ingestion of phosphorus significantly lowered plasma glucose by ~5 mg/dL ($p = 0.016$) and reduced insulin levels by ~20% at 60 minutes ($p = 0.002$), leading to an improvement in the insulin sensitivity index ($p < 0.006$). In a second experiment, where phosphorus was ingested one hour

prior to glucose, no significant differences in glucose or insulin were observed between treatments.

Collectively, these findings indicate that while most studies found no effect of phosphorus supplementation on postprandial glucose or insulin when measured at 2-hour intervals or longer, the more detailed sampling protocol used by Khattab et al. (2015) revealed significant acute improvements in glycemic and insulinemic responses when phosphorus was co-ingested with glucose.

2.4.5 Phosphorus, body composition, and satiety

Three studies investigated the effects of dietary phosphorus on appetite regulation, energy intake, and body weight **(Table 2.2)**, with phosphorus doses ranging from 375 mg to 500 mg per meal. Bassil et al. (2016) examined adults who consumed a 75 g glucose solution with or without 500 mg phosphorus. Appetite ratings, including satiety, hunger, and desire to eat, were measured using visual analog scales (VAS) at fasting and at 30-minute intervals for 3 hours post-ingestion. Phosphorus supplementation significantly decreased overall appetite scores by 14% in overweight/obese participants ($p = 0.02$) and increased diet-induced thermogenesis by 23% compared with glucose alone ($p < 0.05$).

Obeid et al. (2010) tested the effect of phosphorus added to preload solutions (50 g of sucrose, fructose, glucose, or water) in 53 healthy adults. Each preload was given with or without 500 mg phosphorus, followed by an ad libitum lunch. Energy intake at the subsequent meal was reduced by 27% when phosphorus was added to the water preload, and by 33–35% when added to sucrose or fructose preloads, demonstrating a clear inverse

relationship between phosphorus content of the preload and caloric intake ($p < 0.05$). Postprandial satiety ratings were not explicitly reported in this study, but the reduction in subsequent energy intake indicates enhanced subjective fullness.

Ayoub et al. (2015) conducted a 12-week intervention in overweight and obese adults, administering 375 mg phosphorus per meal with breakfast, lunch, and dinner. Body weight decreased by 0.65 kg in the phosphorus group versus an increase of 1.13 kg in the placebo group ($p = 0.01$). Waist circumference was reduced by 3.62 cm relative to placebo ($p < 0.001$). Appetite ratings, measured by VAS, showed significant reductions in hunger, quantity of food needed to reach fullness, taste-related desire, and snack frequency in the phosphorus group compared with placebo ($p < 0.05$). No significant changes were observed in fasting or postprandial glucose, insulin, or lipid parameters, indicating that the effects on energy intake and body composition were independent of major postprandial metabolic shifts.

2.4.6 Assessment of risk of bias

Of the nine included studies, one was rated as having low risk of bias, for all five assessment domains (**Table 2.3**). Five were rated as having an unclear risk of bias because no explicit information was provided on blinding and randomization process, nor on the extent of missing data, in addition to some identified concerns regarding the selection of reported results. For instance, one of the studies had few missing reported outcomes in both placebo and phosphorus groups, yet no explanation was given for the reason of this missing data (Ayoub et al, 2015). Three studies were rated as having high risk of bias, owing to the lack of information on participants' blinding and randomization process.

Table 2.3. Risk of bias of included studies, according to the Revised Cochrane risk of bias tool for randomized trials.

Study, year	Experimental	Comparator	Outcome	D1	DS	D2	D3	D4	D5	TOTAL
Abdouni, 2018	Protein meal +/- P	High protein meal +/- P	Energy expenditure							
Assaad, 2018	Obese adults +/- P	Lean adults +/- P	Energy expenditure							
Basil, 2016	Lean adults: glucose solution +/- P	Obese adults: glucose solution +/- P	Energy expenditure, postprandial satiety							
Ayoub, 2015	Test meal with P	Test meal with placebo	Lipidemia, glycemia, weight gain, WC							
Hazim, 2014	High fat meal with P	High fat meal with placebo	Lipidemia, glycemia							
Khattab, 2015	Glucose solution with P	Glucose solution with placebo	Glycemia							
Obeid, 2010	Test meal with P	Test meal with placebo	Energy intake							
Shuto, 2009	Test meal with 1200 mg P	Test meal with 400 mg P	Endothelial function, glycemia							
Volk, 2022	Test meal with P	Test meal with placebo	Lipidemia, glycemia							

D1, Randomization process; *DS*, Bias arising from period and carryover effects; *D2*, Deviations from the intended interventions; *D3*, Missing outcome data; *D4*, Measurement of the outcome; *D5*, Selection of the reported result;
+/-, with and without; P, Phosphorus.
Low risk; Some concern; High risk

2.5 Discussion

2.5.1 Key findings

The main findings of this review can be summarized as follows 1) there is a clear, established, positive association between phosphorus exposure and diet induced thermogenesis, 2) the impact of phosphorus on postprandial glycemia and lipidemia remains unclear, 3) there is a significant inverse association between phosphorus consumption and weight gain, phosphorus consumption and energy intake, in addition to a positive association between phosphorus consumption and satiety.

2.5.2 Phosphorus and thermogenesis

Studies of this review confirm the significant impact and contribution of phosphorus in diet induced thermogenesis. Abdouni et al. detected a difference in postprandial thermogenesis of healthy adults secondary to phosphorus, irrespective of the protein content of the meal, whereby both normal protein test meals and high protein test meals groups had similar diet induced thermogenesis that was increased following 500 mg ingestion of phosphorus ($p = 0.005$) (Abdouni et al, 2018). Assaad et al. found that the ingestion of 500 mg of phosphorus with a high carbohydrate meal enhanced postprandial energy metabolism on average by 0.06 kcal/min in both lean and obese subjects ($p < 0.01$), as measured by substrate oxidation (Assaad et al, 2018). Likewise, Bassil et al. found that 500 mg phosphorus supplementation to glucose solutions increased postprandial thermogenesis in both lean and obese adults, as compared to placebo groups (Bassil et al, 2016). The effect was more pronounced among subjects living with obesity, where phosphorus supplementation induced a 23% increase in their diet induced thermogenesis area under the curve ($p < 0.05$).

Our findings can be explained as follows. Firstly, it is well established that metabolites' supply and availability from exogenous meals impact the rate of ATP production (Kyriazis, 2022), mainly by increasing the cellular uptake of substrates required for its synthesis – particularly glucose and phosphorus (Peterson et al, 2005). In doing so, it is plausible to deduce that phosphorus supplementation with meals triggers insulin release (as a normal postprandial metabolic response), which in turn activates subsequent hepatic uptake of phosphorus, hence increasing ATP production, and diet induced thermogenesis. Hepatic postprandial ATP production, nonetheless, has been strongly associated with signaling satiety, via means of the hepatic vagal afferent activity which detects and transmits to the central nervous system any changes in hepatic energy status, through the stimulation of satiation (Friedman, 2007). This further explains the results of Bassil et al. and Obeid et al., who found that phosphorus supplementation of 500 mg was inversely associated with appetite and the suppression of ad libitum energy intake at subsequent meals (Bassil et al, 2016; Obeid et al, 2010). Likewise, Ayoub et al found that phosphorus loading of 375 mg per meal for 12 weeks, was able to induce significant reduction in changes in appetite, quantity of food to reach fullness, body mass index, waist circumference, and body weight, in overweight and obese adults, as compared to placebo groups (Ayoub et al, 2015).

The underlying mechanism by which phosphorus affected food intake control and body weight is once again related to its direct involvement in optimizing ATP production and improving postprandial energy metabolism. Beyond its immediate role in ATP synthesis, phosphorus may also influence thermogenesis through mitochondrial activation and AMP-activated protein kinase (AMPK) signaling. Increased intracellular

phosphate availability enhances oxidative phosphorylation efficiency and ATP turnover, which in turn stimulates AMPK and uncoupling protein (UCP) activity, promoting greater postprandial energy dissipation as heat (Hardie et al., 2012). These mechanisms align with the observed increases in diet-induced thermogenesis across studies, suggesting that phosphorus facilitates energy flux within hepatocytes and myocytes, thereby enhancing whole-body metabolic efficiency following meal ingestion.

2.5.3 Phosphorus and lipid metabolism

It is hypothesized that phosphorus supplementation would enhance lipid clearance from circulation due to its insulin sensitizing capability, given that the synthesis, clearance, and hydrolysis of triglycerides, chylomicrons and lipoproteins are affected by insulin (Adiels et al, 2008; Duez et al, 2008). In this review however, no significant changes were seen in postprandial lipid parameters between phosphorus and non-phosphorus exposure groups (Ayoub et al, 2015; Hazim et al, 2014; Volk et al, 2022). Our findings differ from other comparable studies that were found in the literature (which were not included in this review, due to incompatibility with inclusion criteria). For example, Ditscheid et al found that 4 weeks of supplementation with pentacalcium hydroxy-triphosphate (CaP) was able to significantly improve overall cholesterol metabolism, whereby: 1) serum cholesterol concentrations were lower than that of placebo ($p = 0.008$), 2) serum LDL cholesterol and the ratio of LDL: HDL cholesterol were lower after CaP supplementation ($p = 0.083$ and $p = 0.062$, respectively), and 3) bile acid excretion was higher among the CaP group ($p = 0.003$). According to the authors, the above observed beneficial effect of phosphorus supplementation on cholesterol metabolism is not related to an increased clearance of cholesterol, but in reality, due to an increased bile acid

excretion and a subsequent formation of bile acids from endogenous cholesterol in the liver (Ditscheid et al, 2005). This is a consequence of dietary calcium and phosphate precipitating in the small intestine to form insoluble amorphous calcium phosphate (ACP), which binds and inactivates luminal bile acids (Ditscheid et al, 2005). Additionally, a pooled review of 21 intervention trials conducted by Trautvetter et al. found that supplementation with calcium phosphate rather than phosphate only in healthy humans resulted in increased bile acid excretion, decreased blood lipids, and modulation of the intestinal environment, once again through ACP formation in the small intestine (Trautvetter et al, 2018). This might explain the results of this review with regards to lipidemia, since the included studies of this review were solely supplemented with phosphorus, overlooking the synergistic role that phosphorus holds when administered with other minerals, especially with calcium.

Beyond intestinal mechanisms, recent experimental evidence highlights a more direct regulatory role of phosphorus in hepatic lipid metabolism. In a rodent model, dietary phosphorus restriction was shown to down-regulate genes involved in hepatic amino acid catabolism and lipogenesis, while up-regulating genes related to fatty acid β-oxidation and energy metabolism (Chun et al, 2016). These findings confirm that phosphorus availability can modulate hepatic metabolic pathways governing lipid synthesis and oxidation, possibly through changes in cellular energy status and ATP-dependent signaling. Although these molecular effects have not been demonstrated in human intervention studies, they provide a mechanistic framework that may underlie phosphorus-induced alterations in lipid metabolism observed under certain experimental or dietary conditions.

It is plausible that similar energy-dependent regulatory mechanisms operate in humans, given the shared metabolic reliance on ATP-driven lipid processing. Hepatic phosphate availability could alter the activity of ATP-citrate lyase and acetyl-CoA carboxylase, key enzymes in de novo lipogenesis, thereby modulating lipid synthesis and export (Pietrocola et al., 2015). Consequently, fluctuations in intracellular phosphorus may shift hepatic metabolism toward greater β-oxidation and reduced lipid accumulation, consistent with the gene expression changes reported in phosphorus-restricted rodents (Chun et al, 2016). These mechanistic insights provide a physiological basis for the inverse associations occasionally observed between dietary phosphorus and plasma lipid concentrations in population studies.

2.5.4 Phosphorus and carbohydrate metabolism

At the glycemic level, although phosphorus partakes a crucial role in insulin signaling and carbohydrate metabolism via the phosphorylation of glucose to glucose-6-phosphate, no clear association between phosphorus supplementation and postprandial glycemia can be deduced from this review. It should be noted that three of the four studies that showed no association were not specifically designed to test for the effect of dietary phosphorus on glycemia. These studies might have failed to detect any association between phosphorus exposure and postprandial glycemia, since they have measured serum glucose at baseline and/or 120 minutes post-meal ingestion only, while overlooking the fact that plasma glucose concentrations typically begin to rise at 10 minutes post meal ingestion, peak at 60 minutes, and return to pre-prandial levels within 120 to 180 minutes (American Diabetes Association, 2001). Heterogeneity of our results with regards to glycemia are in accordance with other studies found

in the literature. Haap et al found that low serum phosphate levels are associated with high 2-hour blood glucose levels (p = 0.130) and reduced insulin sensitivity (p = 0.0006) in healthy adults, with no effect on insulin secretion levels (Haap et al, 2006). In this case, phosphorus supplementation would by default increase postprandial serum phosphate levels, yet whether it would reverse the identified hyperglycemic parameters or not, remains unclear. In contrast to the above, a large prospective E3N cohort study revealed that a high dietary phosphorus intake (>1700 mg/day) was associated with an increased risk of type 2 diabetes (Mancini et al, 2018). Animal studies investigating the association between phosphorus supplementation and glycemia however, reported more conclusive and favorable results. An animal study found that mice on a low-phosphate diet had a 4-fold increase in insulin resistance measured by homeostatic model assessment (HOMA) (p = 0.005), as compared to mice fed high-phosphate diets (Ellam et al, 2011). Additionally, it was found that phosphorus supplementation improved HOMA-insulin resistance in db-db mice after 8 weeks (Eller et al, 2011), and lowered plasma insulin levels in healthy rats after 4 weeks (Abuduli et al, 2016). Mechanistically, low phosphate availability may further impair hepatic energy status, disrupting glycogen synthesis and promoting compensatory gluconeogenesis (Ellam et al., 2011). These processes could contribute to the elevated postprandial glucose and insulin levels observed in low-phosphate states in both animal models and certain human cohorts, providing additional mechanistic context despite the inconsistent findings in intervention trials. All in all, it may be concluded that the direct effect of phosphorus supplementation on glucose metabolism remains uncertain, rather than unlikely, and that low serum phosphate levels can at the very least disrupt glucose homeostasis and insulin sensitivity, even in otherwise healthy subjects.

2.5.5 Strengths and limitations

This systematic review was stringently conducted following the PRISMA guidelines, which ensured a transparent and comprehensive process. To minimize errors and avoid study selection biases, full-text screening was performed by two independent reviewers. This dual-screening approach was implemented to enhance the accuracy and reliability of the included studies. In addition, a well-defined and controlled set of vocabulary was applied, which included structured synonyms and carefully selected title and abstract search terms. This approach facilitated the effective identification of both indexed and non-indexed papers. Given the rigor of our search methodology, we are confident that had the studies designed to address this specific research question been published, they would have most likely been captured in our search. Furthermore, a formal quality assessment was conducted on all nine included studies using the revised *Cochrane Risk of Bias Tool for Randomized Trials*. This thorough assessment further strengthens the credibility of this review.

Despite the strengths of this review, several potential limitations should be considered. First, the search was limited to only three databases, excluding other relevant sources such as Scopus, EMBASE, and ISI Web of Science. The omission of these additional databases may have led to the exclusion of potentially relevant studies, limiting the comprehensiveness of the search. Second, the search was specifically aimed at identifying studies that focused solely on phosphorus supplementation as the primary intervention. This focus allowed for the evaluation of the independent effect of dietary phosphorus, distinct from other minerals. As a result, studies investigating phosphorus in combination with other minerals such as calcium, potassium, and magnesium were excluded. These minerals are physiologically

interrelated, and their inclusion would have made it challenging to assess the distinct role of phosphorus. While this exclusion helped clarify the independent effects of phosphorus, it narrowed the scope of the review.

Third, at the experimental level, many of the included studies had small sample sizes, which limited their reliability and generalizability. This issue is especially notable in the absence of power calculations in some studies, which would have provided additional insights into the robustness of the findings. Additionally, all of the studies included were crossover trials, with no parallel-design studies. This design limitation means that carryover effects between the phosphorus exposure and non-exposure groups cannot be entirely ruled out, potentially influencing the interpretation of the results.

2.6 Conclusion

Put together, this systematic review demonstrated a clear, established, positive association between phosphorus exposure and diet induced thermogenesis, independent of glucose and lipid metabolism. A significant inverse association between phosphorus intake and weight gain, energy intake, and satiety was also detected. No consistent association was identified between dietary phosphorus and glycemia, nor with lipidemia. Although findings from this study may be promising in the world of obesity and metabolic syndrome management, more specific studies are warranted to confirm the triangular association between dietary phosphorus, postprandial metabolism, and glycemic control, and to further identify the underlying physiological mechanisms that bridge them together. If further supported, results of this study put emphasis on the importance of maintaining optimal phosphorus intake levels –either from food or from supplementation at moderate levels–, for the primary prevention of components of the metabolic syndrome in the general population. This in turn, could be achieved via the establishment of a recommended mineral to energy ratio, for instance, mg of phosphorus per kcal of ingested energy, which shall remain far below the tolerable upper level of phosphorus intake.

Chapter 3. The Impact of Dietary Phosphorus on Postprandial Glycemia and Thermogenesis in Healthy Individuals: Evidence from Controlled Clinical Trials

3.1 Introduction

Dietary phosphorus is a key nutrient involved in energy metabolism, ATP synthesis, and glucose regulation. Despite its essential role in metabolic processes, the acute effects of phosphorus intake on postprandial glycemia, diet-induced thermogenesis (DIT), and appetite regulation remain incompletely understood. Controlled clinical trials provide a unique opportunity to investigate these effects under tightly monitored conditions, offering mechanistic insights that complement observational findings and systematic evidence. This chapter presents two human experiments designed to evaluate the influence of dietary phosphorus on postprandial metabolic responses in healthy adults. The studies collectively explore both inorganic phosphorus supplementation and naturally occurring phosphorus in dairy, enabling a comparison of different sources and forms of phosphorus on glucose handling and energy metabolism.

This project was conducted in Beirut, Lebanon, from October 2023 to November 2024, in collaboration between the University of Nottingham in the UK and the American University of Beirut. It comprised two controlled human research trials, each adhering to the same overarching protocol, design, and methodology, while investigating distinct interventions. The first commenced in November 2023 and ended in February 2024, while the second began in August 2024 and ended in November 2024. The participant cohorts for each trial were recruited separately and will be described in detail in the relevant sections below.

3.2 Objective

The overall aim of this dual study is to clinically evaluate the impact of dietary phosphorus on carbohydrate metabolism and diet induced thermogenesis, via the continuous monitoring of blood glucose levels and core body temperature. The specific objectives of each experiment, along with their expected outcomes, are presented in **Figure 3.1.**

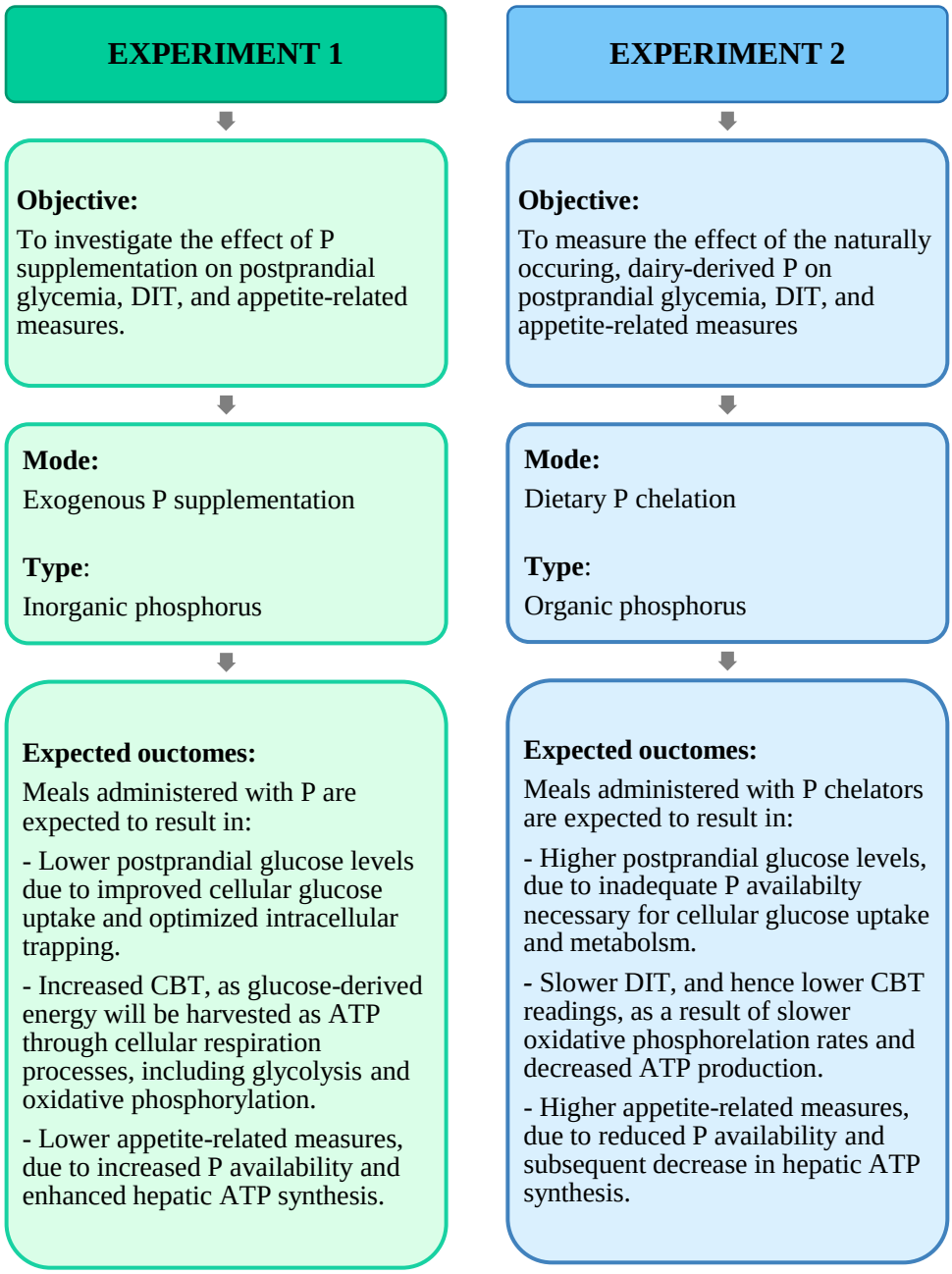


Figure 3.1. Summary of objectives and related expected outcomes.

P, phosphorus; DIT, diet-induced thermogenesis; PEE, postprandial energy expenditure; ATP, adenosine triphosphate; EE, energy expenditure; CBT, core body temperature.

3.3 Methodology

3.3.1 Ethical considerations

The protocol of this project was approved by the Institutional Review Board (IRB) of the American University of Beirut on March 9, 2023, under the identification number BIO-2022-0015, any by the Faculty of Medicine and Health Sciences Research Ethics Committee of the University of Nottingham on April 6, 2023, under the ethics reference number FMHS 392-1121. Both experiments were conducted in accordance with the guidelines laid down in the Declaration of Helsinki. Written informed consents were obtained from all subjects prior to their participation in any of the two studies (**Appendices C and D)**.

3.3.2 Study design and power analysis

Both experiments were single-blinded, randomized placebo-controlled crossover studies, where participants served as their own control. Based on data from Westerterp-platenga et al, the mean postprandial elevation in body temperature is 0.8°C (SD = 0.35) (Westerterp-platenga et al, 1990). Accordingly, each of the two experiments required a sample size of 16 participants (8 males and 8 females) to achieve adequate statistical power. The probability that this study would detect a treatment difference at a two-sided 0.05 significance level is 90 percent, if the true difference between treatments is 0.3°C (a difference of 40% between groups or treatments). This is based on the assumption that the within-patient standard deviation of the response variable is 0.35.

3.3.3 Recruitment and eligibility

Students and staff of The American University of Beirut were recruited for this research project at two separate time-points: November 2023 for enrollment in Experiment 1, and August 2024 for enrollment in Experiment 2. Participants' recruitment process is outlined in **Figure 3.2.** Both studies were advertised using posters located within university departments, leaflets, word of mouth, and emails distributed throughout the university (**Appendix E**). Potential subjects who expressed interest in either study were subjected to a preliminary eligibility assessment. It included questions regarding their overall health status, use of medications, and dietary restrictions. This assessment was carried out using a pre-screening evaluation questionnaire administered by the study personnel (**Appendix F**).

A detailed overview of the inclusion and exclusion criteria employed for participant selection, for both experiments, is listed in **Table 3.1.** Candidates who met the initial inclusion criteria (N = 16 for each experiment) were scheduled for a screening visit, during which their anthropometric measurements and body composition analyses were recorded. Height measurements were taken using a portable stadiometer. Weight and body composition were assessed utilizing the *InBody 770* machine (Cerritos, California). A random non-fasting finger-prick blood glucose test was conducted to identify any potential pre-diabetes or diabetes conditions. The calibrated *One Touch* glucose strip was used for this purpose, and a blood glucose level reading of less than 180 mg/dl was considered within the normal range. Additionally, a urine sample was collected to assess renal function and detect albuminuria. The samples were analyzed for urine albumin-to-creatinine ratio (uACR) at the AUBMC

Pathology Lab Medicine Department (PLM). Normal levels were determined at an albumin-to-creatinine ratio of less than 30 mg/g.

The screening visit lasted for approximately 20 minutes, after which candidates received clear and comprehensive information about the study's timeline, protocol, as well as the potential risks, benefits, and discomforts associated with participation. Interested and eligible participants could choose to participate in one or both experiments. Participation was voluntary and written informed consent forms were obtained from all participants.

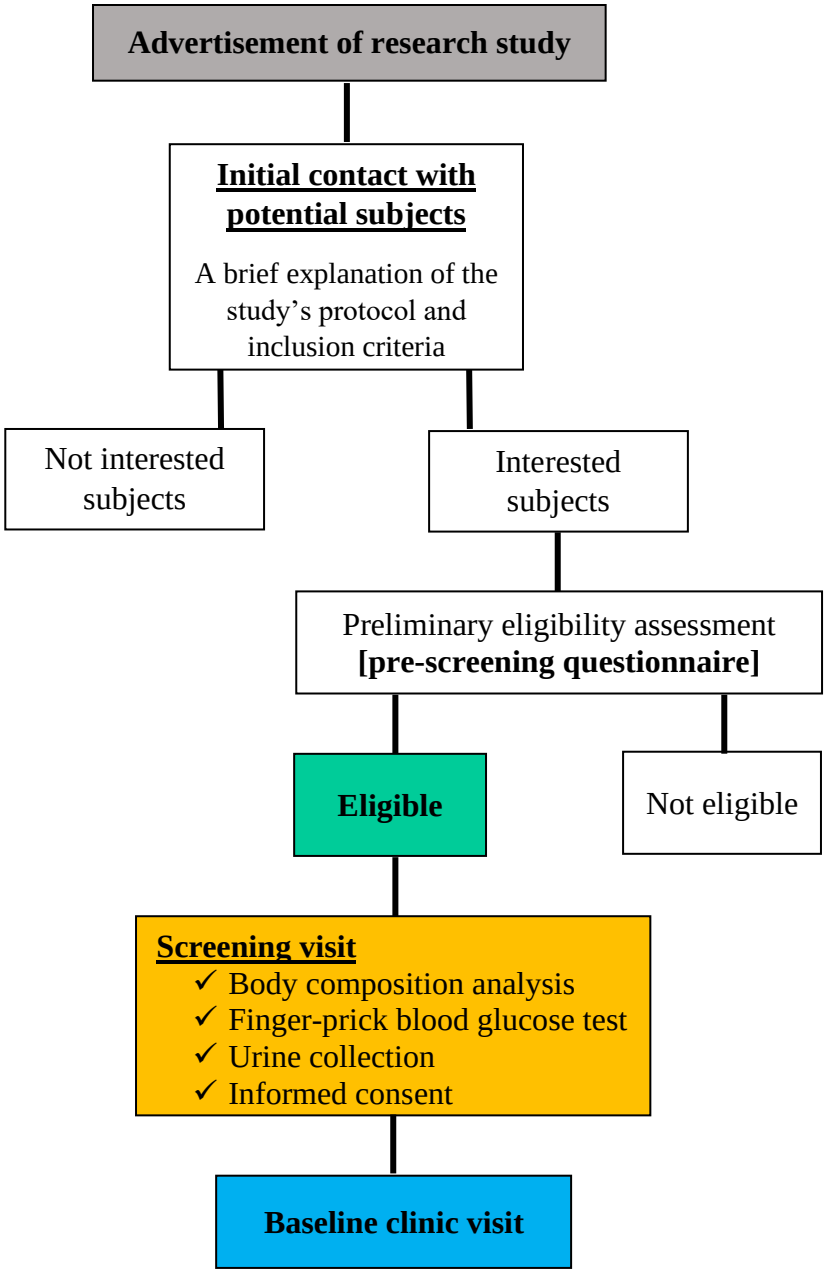


Figure 3.2. Flowchart of recruitment process for both experiments.

Table 3.1. Participants' inclusion and exclusion criteria for both experiments.

Preliminary Inclusion Criteria	Preliminary Exclusion Criteria
Adults aged 18 to 64 years	Pregnant or lactating
Normal BMI (19.5 – 24.5 kg/m^2)	Overweight or obese
Willing and able to give informed consent for participation in the study.	Have prediabetes, diabetes, or renal failure.
Not taking any medications or vitamin/mineral supplements that might affect body weight, glucose, or lipid metabolism.	Have history of chronic diseases, renal, endocrine, hepatic, thyroid, or cardiac diseases.
Willing to maintain his/her regular dietary and physical activity habits throughout the 10-day study period.	Have food allergies or intolerance to milk and dairy products.
Willing to avoid alcohol consumption as well as any unusual strenuous activity, 24 hours prior to each experimental session.	Have history of bariatric surgeries.
Have a smartphone and capable of understanding how to use continuous glucose monitoring and CBT devices with their synchronized mobile applications.	Have history of kidney stones.
Have the Health Insurance Plan (HIP) coverage provided by AUB.	Have history or current psychiatric illness.
Have no intentions of travelling during the 10 days study period, due to daily research commitments.	Have experienced weight loss of 3% or more in the preceding 3 months.

BMI, body mass index; CBT, core body temperature; AUB, American University of Beirut.

3.3.4 Continuous monitoring devices

To serve the objective of this research project, participants of both experiments were asked to wear 2 devices throughout the entire study period: a continuous body temperature monitoring device and a continuous glucose monitoring device.

3.3.4.1 Continuous body temperature monitoring

Core body temperature was monitored using the CORE Research System (greenTEG, Zurich, Switzerland), a non-invasive, wearable waterproof device that provides continuous, minute-by-minute measurements of core and skin temperatures through integrated thermal sensors (CORE, 2020). The CORE device has been applied in various research settings, including exercise physiology, thermoregulation during endurance sports, and everyday living conditions, demonstrating its utility for continuous metabolic monitoring (Verdel et al, 2021; Etienne et al, 2023;). The CORE device's accuracy has been evaluated under controlled conditions, with electronic pills used as a benchmark for comparison (CORE Accuracy, 2020), as illustrated in **Figure 3.3**.

While the device has been widely used, recent studies have highlighted potential limitations in its ability to precisely reflect absolute core body temperature under certain conditions (McLaughlin et al, 2025; Desroches et al, 2023; Goods et al, 2023; Verdel et al, 2021;). Despite these limitations, the CORE system was considered the most feasible and practical option for the current study, which required continuous monitoring of postprandial thermogenic responses alongside glucose levels. Alternative methods, such as indirect calorimetry or ingestible temperature pills, would have been

impractical for the continuous 10-day protocol, due to participant burden, cost, and the inability to capture minute-by-minute temperature fluctuations across multiple postprandial periods. Moreover, its non-invasive design ensured participant comfort and compliance throughout the intervention, while providing data that could be directly synchronized with continuous glucose monitoring.

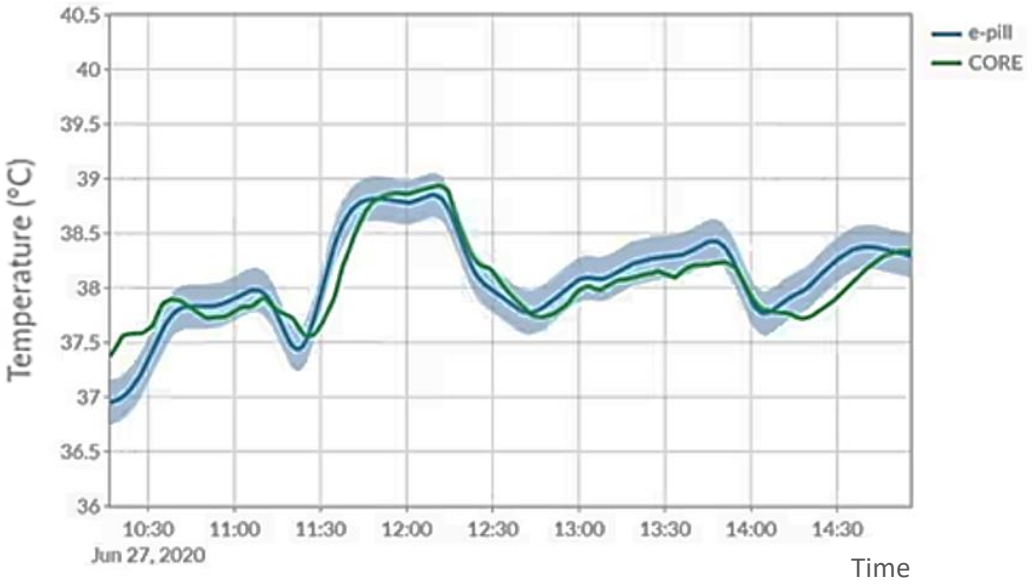


Figure 3.3. Comparison of core body temperature measurements using the CORE device and ingestible electronic pill.

Core body temperature was monitored simultaneously using an ingestible electronic pill (e-pill) and the non-invasive CORE wearable device during an outdoor cycling session in winter conditions (mountain ride, ambient temperature 10–15 °C). This comparison highlights the agreement between the two measurement methods under real-world conditions.

Adapted from *CORE Accuracy*, 2020.

3.3.4.2 Continuous glucose monitoring

Serum glucose concentrations were monitored using the Dexcom G6 System (San Diego, California), which provides integrated continuous glucose monitoring (iCGM) readings updated every 5 minutes, delivering real-time glucose levels and trends (DEXCOM, 2019). The Dexcom G6 System is FDA-approved and authorized as a standalone glucose determination device for individuals aged two years and above, offering a suitable alternative to finger-stick testing (DEXCOM, 2019). The system demonstrates exceptional accuracy, with an overall Mean Absolute Relative Difference (MARD) of 9% (Wadwa et al, 2018), and has been shown to perform consistently even during rapid glucose fluctuations (Shah et al., 2018). Its accuracy has also been evaluated in non-critically ill hospitalized patients with diabetes, mild to moderate anemia, cardiovascular and respiratory illnesses, and impaired renal function, with a MARD of 12.8% and median Absolute Relative Difference (ARD) of 10.1% (Davis et al, 2021). The device is non-invasive, water-resistant, and consists of three main components: a sensor, a Bluetooth Low Energy (BLE) transmitter, and a BLE-enabled receiver.

Despite these strengths, CGM has known limitations. These include a potential lag between interstitial and plasma glucose levels, calibration requirements, and sensitivity to sensor placement or local tissue factors, which may impact absolute accuracy. Notably, interstitial glucose readings can lag behind capillary blood glucose readings by as much as 20 minutes, especially during periods of rapid glucose fluctuations such as after meals or physical activity (Sun et al, 2021; Zaharieva et al, 2019). Additionally, sensor accuracy can be influenced by factors such as insertion site, local tissue perfusion, hydration status, and calibration procedures (Sun et al,

2021). Furthermore, the sensor's sensitivity may decrease over time due to biofouling, a process where the body's immune response encapsulates the sensor, leading to reduced performance (Sun et al, 2021).

Nonetheless, CGM was chosen for this study due to its feasibility within a continuous, multi-day experimental protocol. Traditional laboratory-based postprandial glucose assessments, such as frequent venous sampling, would have been more invasive and burdensome, while less practical for capturing real-world fluctuations. The continuous nature of CGM allowed for synchronized monitoring alongside core body temperature measurements and dietary interventions, providing a practical balance between data resolution, participant compliance, and overall study logistics.

3.3.5 Study protocol

Both experiments followed an identical protocol (**Figure 3.4)**, with the key difference lying in the type of intervention/test meal administered. In Experiment 1, participants received a refined carbohydrate meal, with and without phosphorus supplementation, while in Experiment 2, participants were provided with a dairy drink (constituted of full-fat powdered milk), with and without phosphate chelation. The standardized test meals provided for each experiment are summarized in **Table 3.2**, with the respective ingredients and nutrient composition described extensively in section 3.2.5.

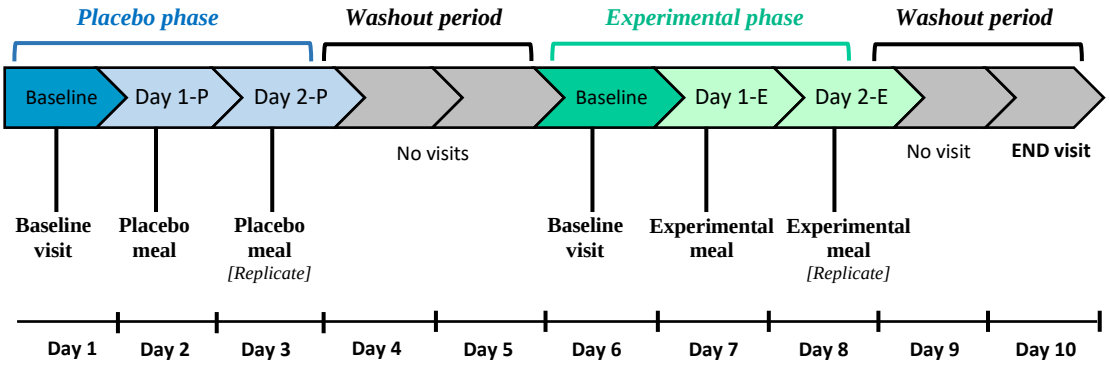


Figure 3.4. Protocol and timeline flowchart of both experiments.

P, placebo; E, experimental.

Each experiment spanned 10 consecutive days and consisted of two interventional phases: a placebo/control phase and an experimental phase, separated by a 2-day washout period to mitigate potential carryover effects. Each of the phases began with a baseline visit, followed by 2 days of interventional visits, during which participants consumed test meals (either placebo or experimental meal), as illustrated in **Figure 3.4**. The assignment of each participant to his/her respective intervention (i.e. placebo or experimental) was random. On the two interventional days –referred to as Day 1 and Day 2 of either the placebo or experimental phase– participants consumed the same test meal on both days, with Day 2 serving as an exact replicate of Day 1. For instance, if a participant was administered a refined carbohydrate meal with phosphorus supplements on Day 1, he/she will receive the exact same meal on Day 2. This approach was implemented to enhance consistency, reproducibility, and validity of the results, ensuring that the observed outcomes were robust and not attributable to random variability.

Table 3.2. Standardized test meals provided for each experiment.

	STANDARDIZED TEST MEAL	
	Placebo meal	***Experimental meal***
EXPERIMENT 1 ***(Phosphorus supplementation)***	Refined carbohydrate meal with 6 phosphorus-free placebo tablets.	Refined carbohydrate meal with 6 oral potassium phosphate tablets (equivalent to 684 mg of phosphorus).
EXPERIMENT 2 ***(Dairy phosphorus chelation)***	Full fat powdered milk with one phosphorus-free placebo tablet.	Full fat powdered milk with one phosphate binding tablet.

3.3.5.1 Baseline visits

During the initial baseline visit, the study staff conducted one-on-one sessions with the participants to explain the purpose of wearable devices, as well as the overall compliance requirements. The visit started with instructing the participants to download the 'CORE – Temperature Monitor' mobile application on their smartphones, which is compatible with both IOS and Android devices. Individual accounts were established using the participants' email addresses and the CORE device was paired with their accounts via Bluetooth transmission to facilitate data collection. Following the cleaning and drying of the designated area, the device was attached to the upper right side of the chest, near the liver, using adhesive pads. **Figure 3.5** illustrates a representative image depicting the location of the CORE device. The CORE body temperature monitoring device features a 3-day battery life for continuous data transmission and is validated with a high accuracy of ± 0.21°C (*CORE Accuracy*, 2020). Guidance on the use of the application was provided to participants. They were also instructed to maintain the device in its designated position for optimal temperature recording. The research team had access to the CORE device measurements, allowing them to promptly identify any abnormal values and ensure that the devices were correctly positioned. A CORE device charger was provided to participants for use during the washout period (days 4 and 5).

Following the CORE device insertion, trained study staff activated and set up the Dexcom receiver using specific sensor and transmitter codes. The glucose alert levels were set at 70 mg/dl and 180 mg/dl, as the low and high threshold limits, respectively. To ensure a seamless insertion process, areas with scaring, tattoos, hair or irritation were avoided. The insertion process

began with cleaning the insertion site using alcohol pads. Using a one-touch applicator, a slim sensor was subcutaneously inserted into the lower right quadrant of the abdomen. **Figure 3.5** demonstrates a representative image of the exact location of the Dexcom G6 device. This slim sensor continuously monitored glucose levels and wirelessly transmitted the data to a compatible receiver, via a transmitter, which was also attached to the sensor on the participant's body. Participants were instructed to keep the sensor and transmitter in place throughout the 10-day study period, while being able to bathe or shower normally.

The insertion process was simple and painless. In a previous clinical study testing the Dexcom G6 device, users' feedback indicated that the auto-applicator was "very easy" to use, and 84% reported experiencing either no pain or mild discomfort (Shah et al, 2018). It is noteworthy that none of the participants, in either experiment, reported any pain or discomfort related to the device, neither during insertion, nor throughout the 10-day experimental period. Following its installment, the transmitter was paired with a receiver, a small phone-like device that was given to participants to receive and monitor their own glucose readings throughout the study period. It was imperative that participants kept the receiver within 6 meters of proximity, without obstacles such as walls or metal interfering. A receiver charger was also provided to participants, allowing them to recharge the device whenever the battery level dropped below 50%. The study personnel were able to monitor glucose readings through the Dexcom Clarity application, and in instances of abnormal values, participants received follow up messages (**Appendix G**).

During their first baseline visit, participants were also provided with a comprehensive explanation of the essential details related to the two

wearable devices (**Appendix H).** They were instructed and trained to diligently maintain records of their daily food intake, precise timings of consumption, and quantities of ingredients, throughout the entire study period. Furthermore, participants were required to maintain records of their physical activity, in addition to their sleep and wake times. To maintain anonymity, a unique identification number was assigned to each participant at the end of his/her baseline visit.

At their second baseline visit (Day 6 of the experiment), participants returned to the clinic for the reattachment of the CORE device to their chest (following its removal during the washout period for recharging purposes), ensuring accurate recording of data in the mobile application.

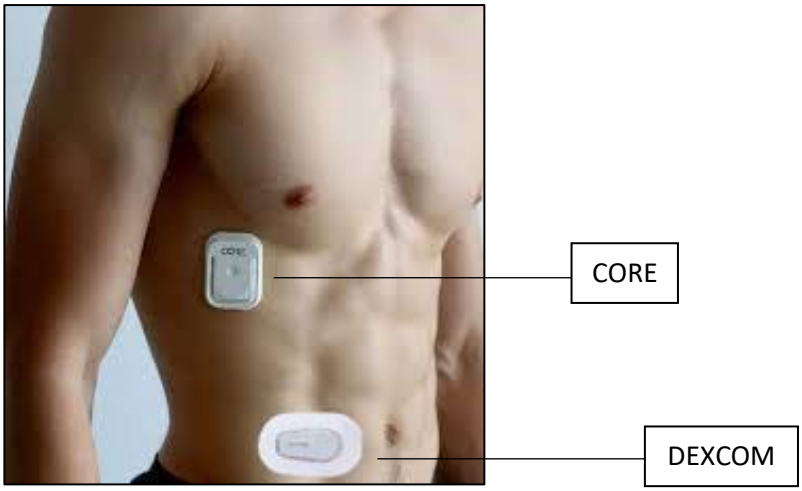


Figure 3.5. Representative image of CORE and Dexcom G6 devices on a participant's body.

3.3.5.2 Interventional visits

Each participant attended 4 interventional visits (Days 2, 3, 7, and 8) –two during the placebo phase and two during the experimental phase– as illustrated in **Figure 3.5**. For each interventional visit, participants were asked to come following a 12-hour overnight fast. On their visit, participants consumed their randomly assigned test meal (placebo or experimental) while wearing the continuous glucose and temperature monitoring devices. They were asked to stay sedentary and refrain from eating for 3 hours post ingestion of every test meal, after which they may eat ad libitum. To gain insights into their appetite, subjective appetite scores were collected from all participants, using validated visual analogue scale (VAS) questionnaires, before, during, and at several time points post-meal ingestion.

3.3.5.3 Washout and end visits

Participants were not required to attend clinical visits during washout periods (Days 4, 5, and 9), instead, they were instructed to continue documenting their dietary intake, physical activity, and sleep/wake schedules. On Day 10 (end visit), participants returned to the clinic, with their Dexcom receiver and device chargers.

3.3.6 Data collection

Following participants' end visit, the recorded data extracted from each CORE device was uploaded from the CORE cloud dashboard, while Dexcom data was uploaded through the Dexcom Uploader Clarity platform. Subsequently, 10-day datasets for both CORE and Dexcom data were generated in Excel format. Interested participants were provided with a summary of their daily glucose levels. Following data upload and verification for accurate dates and measurements, participants were instructed to remove the CORE device and unpair it from the phone application. Participants were also guided to remove the Dexcom slim sensor.

The 10-day uploaded datasets from both CORE dashboard and Dexcom Clarity platform underwent data cleaning procedures in Excel prior to analysis. CBT measurements were recorded on a per-minute basis, while glucose readings were measured every 5 minutes. Consequently, specific formulas were applied in Microsoft Excel to synchronize the 'minute' intervals of CBT readings with those of glucose, ensuring a uniform recording interval of 5 minutes for both variables.

"=AND(MOD(MINUTE(C2),5)=0" and "=MOD(F2,5)=0"

Graphical illustrations of a typical 24-hour trend in glucose, CBT, and skin temperature, for a participant consuming phosphorus and placebo meals, are presented in **Figure 3.6** and **Figure 3.7**, respectively.

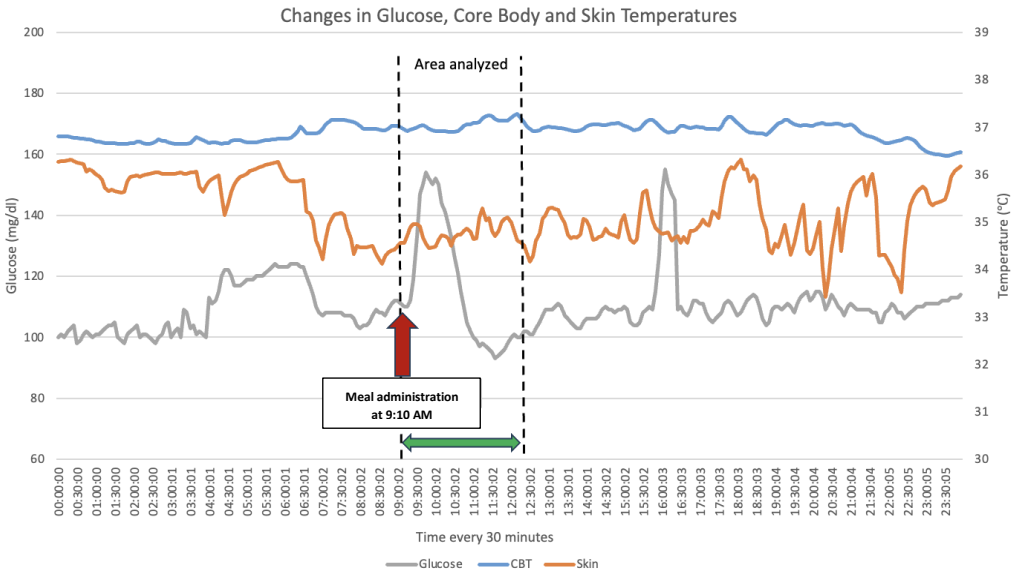


Figure 3.6. Representative multivariate line graph of a 24-hour trend in glucose, core body temperature (CBT), and skin temperature data, for a female participant in Experiment 1, consuming an experimental meal (with phosphorus tablets).

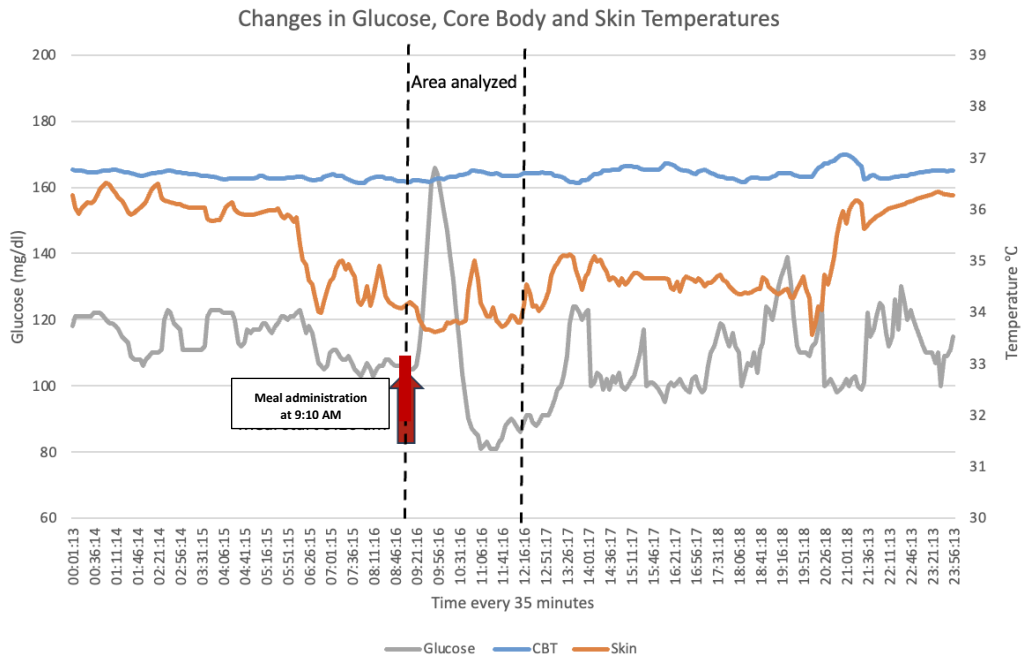


Figure 3.7. Representative multivariate line graph of a 24-hour trend in glucose, core body temperature (CBT), and skin temperature data, for a female participant in Experiment 1, consuming a placebo meal.

3.3.7 Dietary monitoring

Throughout the 10-day experimental period, participants' habitual dietary intake was systematically recorded using standardized 10-day food record sheets (**Appendix I**). Participants were instructed to maintain their usual dietary patterns, refrain from consuming high-phosphorus supplements or foods outside the study protocol, and document any deviations from their typical intake. These records provided a comprehensive account of all foods and beverages consumed, allowing the research team to monitor adherence to study requirements and ensure consistency across participants. Although no formal nutrient analysis was conducted, such that total energy intake, macronutrient composition, and phosphorus content were not quantified, the detailed food records served as a critical tool to assess compliance, minimize potential confounding from unrecorded dietary changes, and support the internal validity of the experimental interventions. The structured monitoring approach also facilitated the identification of any deviations from habitual intake that could influence postprandial outcomes, ensuring that observed effects could be attributed primarily to the experimental manipulations rather than variability in participants' diets.

3.3.8 Standardized test meals

The standardized test meals offered during the placebo and experimental phases of each experiment were isocaloric and underwent the same method of preparation. The only difference in experimental versus placebo meals, was in their phosphorus content, as determined by the use of phosphate supplements, phosphate binders, or phosphorus-free placebo tablets. Meals were prepared in the Nutrition Metabolic Lab kitchen at the Department of Nutrition and Food Sciences, in the American University of Beirut.

3.3.8.1 Test meal of Experiment 1

Participants were administered a refined carbohydrate meal, providing 625 calories in a volume of 250 ml, along with either 6 potassium phosphate tablets (brand name: *K-Phos® Original*) or 6 cellulose-based, phosphorus-free, placebo tablets, in a random order. Both tablets had similar size, shape, and color. Each potassium phosphate tablet provides approximately 114 mg of phosphorus, resulting in an additional 684 mg of phosphorus to every test meal.

The standardized test meal administered in this experiment was intended to be low in phosphorus. Its naturally occurring phosphorus content was approximately 55 mg, since the ingredients used in its preparation (water, sugar, egg white, and corn oil) retain negligible amounts of phosphorus. The ingredients and nutrient composition of the test meal are outlined in **Table 3.3**, and the energy contribution breakdown is illustrated in **Figure 3.8**. The composition of both potassium phosphate and placebo tablets are listed in **Table 3.4** and **Table 3.5**, respectively.

3.3.8.1.1 Phosphorus supplement and palatability assessment (Experiment 1)

Potassium phosphate was chosen as the phosphorus source for Experiment 1 due to its high bioavailability and excellent acceptability in human studies. This choice aligns with prior research, including the studies summarized in our systematic review (Chapter 2), where seven out of nine experimental trials employed potassium phosphate as the preferred supplementation form. In contrast, alternative phosphorus supplements, such as sodium phosphate, have demonstrated lower tolerability; based on previous work, participants frequently reported a pronounced fish-like taste, which negatively impacted palatability and participant compliance.

To ensure that the supplemented meals were well accepted and that the observed metabolic effects were not confounded by aversive responses, an organoleptic assessment of the test meals was conducted using a Visual Analog Scale (VAS). This evaluation systematically examined the visual appeal, smell, taste, aftertaste and overall palatability of the meals. The results confirmed that participants tolerated the meals well, supporting the validity of the experimental findings and demonstrating that potassium phosphate is a suitable and practical choice for dietary phosphorus interventions in controlled trials.

3.3.8.2 Test meal of Experiment 2

Participants in Experiment 2 received approximately 480 ml of a dairy test drink (125 g of full-fat powdered milk (brand name: *TATRA*®), mixed with 250 ml of water), administered with either one cellulose-based, phosphorus-free, placebo tablet or one phosphate binding tablet. The caloric content of this test meal was comparable to that of Experiment 1, amounting to 620 calories. The standardized test drink administered in this experiment was

intended to be high in phosphorus. Its naturally occurring phosphorus content was approximately 970 mg, as milk is known to be a rich source of phosphorus. The overall nutrient composition and the energy contribution breakdown of the dairy test meal are outlined in **Table 3.3** and **Figure 3.8**, respectively. The nutrition facts panel of the powdered milk used in this experiment is illustrated in **Figure 3.9**.

Sevelamer carbonate (brand name: *Renvela*®) was used as the phosphate binder and was administered simultaneously with every drink (swallowed as whole and not crushed). Each *Renvela* tablet contains 800 mg sevelamer carbonate. The generic drug sevelamer carbonate is an orally administered pharmaceutical, widely approved for the treatment of hyperphosphatemia in patients with end-stage renal disease. The recommended starting dose for adults is one to two 800 mg tablets, administered with each meal, 3 times a day. It consists of crosslinked polymeric amine which chelates dietary phosphate in the gastrointestinal tract, subsequently preventing its absorption, and enhancing its fecal excretion (Biswas et al, 2014).

Although calcium-based and aluminum-based phosphate binders effectively reduce serum phosphorus levels, these agents can lead to hypercalcemia, increased vascular calcification, and have been associated with several hematological, skeletal, and neurological toxicity (Biswas et al, 2014). Sevelamer, however, is a non-metal containing phosphate binder, efficacious in reducing serum phosphate levels without causing any adverse metabolic effects, or changes in bone turnover or mineralization. Mounting evidence from randomized trials and observational studies confirmed its efficacy and safety, in terms of its binding capacity constants (K2) and affinity constants (K1), without eliciting any gastrointestinal side effects or

discomfort (Biswas et al, 2014; Spaia, 2011). The nonmedicinal ingredients of Renvela tablets are listed in **Table 3.6.**

Table 3.3. Ingredients and macronutrient composition per serving of the standardized test meals used in Experiments 1 and 2.

EXPERIMENT 1 *(Refined carbohydrate test meal)*			
Ingredients	**Weight**	**Macronutrient composition**	**Energy (kcal)**
Water	120 ml	-	-
White granulated sugar	75 g (6 tbsp)	75 g carbohydrates	300
Egg white powder	50 g (7 tbsp)	50 g proteins	200
Corn oil	14 g (1 tbsp)	14 g fats	126
Strawberry extract	Few drops	-	-
TOTAL			**626**
EXPERIMENT 2 *(Dairy test meal)*			
Ingredients	**Weight**	**Macronutrient composition**	**Energy (kcal)**
Water	250 ml	-	-
Full-fat powdered milk	125 g		
	-	48 g carbohydrates	192
	-	33 g proteins	132
	-	33 g fats	297
Strawberry extract	Few drops	-	-
TOTAL			**621**

Kcal, kilocalories; tbsp, tablespoon.

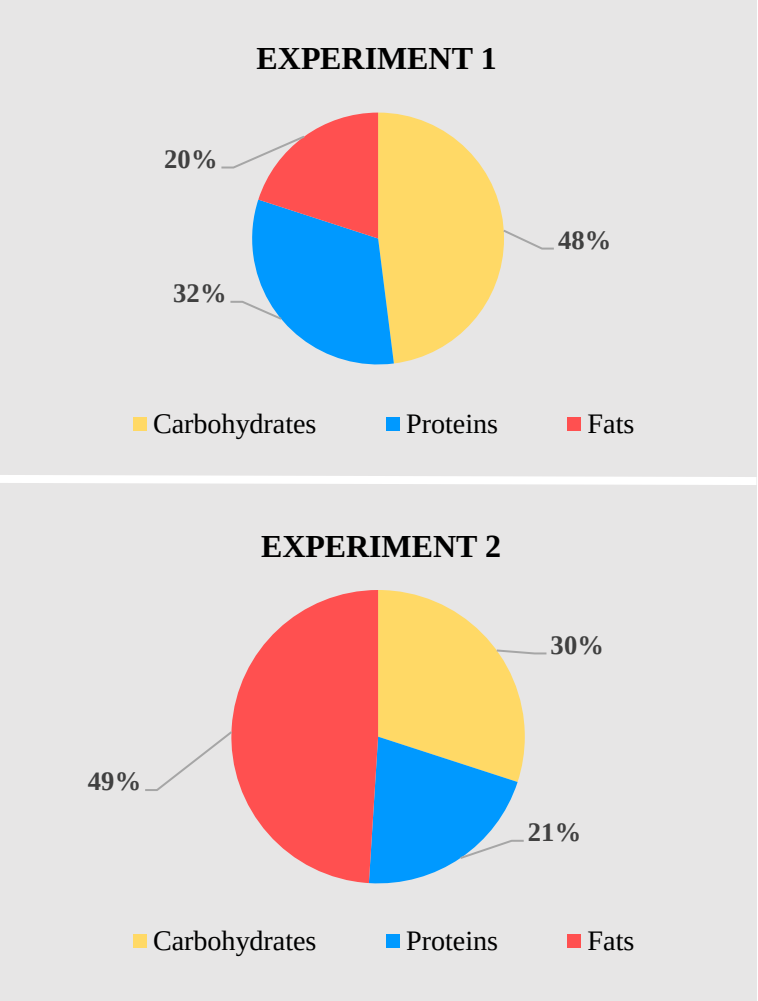


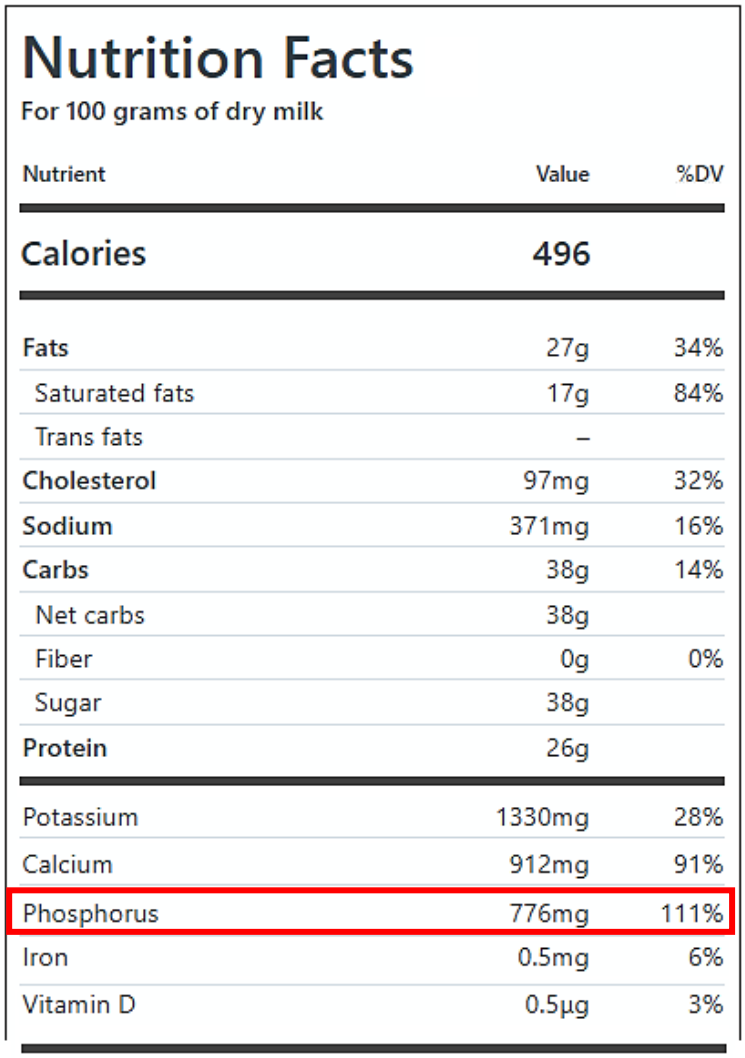
Figure 3.8. Energy contribution breakdown (%) of standardized test meals of Experiments 1 and 2.

Table 3.4. Composition of potassium phosphate (*K-Phos*® *Original*) tablets used in Experiment 1.

Ingredients	Weight (mg)
Potassium phosphate monobasic (KH_2PO_4)	151.52
Potassium phosphate dibasic (K_2HPO_4)	279.6
Dicalcium phosphate	86.4
Micro crystalline cellulose	40
Stearic acid	40
Magnesium stearate	8
Croscarmellose sodium	8
Silicon dioxide	4

Table 3.5. Composition of the placebo capsule used in Experiments 1 and 2.

Ingredients	Weight (mg)
Micro crystalline cellulose	300
Calcium carbonate	200
Stearic acid	160
Magnesium stearate	15
Croscarmellose sodium	20
Silicon dioxide	5



Nutrition Facts

For 100 grams of dry milk

Nutrient	Value	%DV
Calories	**496**	
Fats	27g	34%
Saturated fats	17g	84%
Trans fats	–	
Cholesterol	97mg	32%
Sodium	371mg	16%
Carbs	38g	14%
Net carbs	38g	
Fiber	0g	0%
Sugar	38g	
Protein	26g	
Potassium	1330mg	28%
Calcium	912mg	91%
Phosphorus	776mg	111%
Iron	0.5mg	6%
Vitamin D	0.5µg	3%

Figure 3.9. Nutrition facts panel of TATRA full-fat instant powdered milk.

DV, daily value.

Adapted from Société Tabbara pour le Commerce et l'industrie S.A.L. (2024). *Tatra Instant Powdered Milk.*

Table 3.6. Summary product information of *Renvela*® tablets.

Route of administration	Dosage form/Strength	Nonmedicinal ingredients
Oral	Tablet/ 800 mg	Diacetylated monoglycerides, hypromellose, microcrystalline cellulose, sodium chloride, zinc stearate.

Adapted from *Renvela* Product Monograph, 2023.

3.3.9 Visual Analogue Scale questionnaire

A validated Visual Analog Scale (VAS) questionnaire was used to assess subjective appetite and fullness sensations in both experiments. The self-reported, paper questionnaire consisted of eight horizontal VAS questions, divided into two sections. The first section assessed appetite sensations, measuring hunger, satiety, fullness, and perceived prospective food intake. The second section evaluated food preferences, asking participants to indicate their desire to consume foods that were sweet, salty, savory, or fatty. Each scale was a 10 cm continuous line anchored by opposing descriptors (e.g., not hungry at all to extremely hungry), allowing participants to indicate their perceived state by marking a point along the line. VAS questionnaires are well-validated tools that are widely utilized in appetite research, providing a simple yet sensitive approach to capturing real-time appetite fluctuations (Flint et al, 2000). In 2000, Flint et al. conducted a pivotal study evaluating the reproducibility, power, and validity of these scales in single test meal studies. Their research demonstrated that VAS scores are reliable tools for appetite assessment and are not significantly influenced by prior diet standardization (Flint et al, 2000). The VAS questionnaire used in both experiments is included in **Appendix K**.

3.3.10 Statistical methods

A repeated measures analysis of variance (ANOVA) was performed to assess the effect of phosphorus on each of the outcomes –blood glucose, CBT, and appetite-related parameters. The within-subjects factor was time, with seven levels corresponding to the measured time points: 0, 30, 60, 90, 120, 150, and 180 minutes. The between-subjects factor was treatment group, with two levels (placebo and experimental), and day, with two levels (Day 1 and Day 2). Prior to conducting the repeated measures ANOVA, Mauchly's test of sphericity was used to evaluate the assumption of sphericity. When this assumption was violated, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom. Post-hoc comparisons were performed using Bonferroni-adjusted pairwise comparisons when significant effects were detected. Effect sizes were reported using partial eta squared (η^2) values. For pairwise comparisons, paired t-tests were conducted. Homogeneity of variances was assessed using Levene's test, and the normality of the data was evaluated using the Shapiro-Wilk test, along with visual inspection of histograms. Descriptive statistics, including means and standard errors, were calculated for each time point and treatment group. A significance level of $\alpha = 0.05$ was set for all analyses. The incremental area under the curve (iAUC) for blood glucose was calculated using the trapezoidal rule and expressed in mg·min/dl. Both positive and negative iAUC values were reported to capture the complete postprandial glycemic response, including glucose excursions above baseline (as this reflects the postprandial rise in glucose levels) and subsequent declines below baseline. Data management was performed in *Microsoft Excel*, and statistical analyses were conducted using *SPSS* (version 26; IBM, Armonk, NY, USA).

3.4 Results (Experiment 1)

3.4.1 Subject characteristics

This experiment included a total of 16 participants (8 males and 8 females), all of whom met the established inclusion criteria. Participants had normal BMI values, glucose levels, and kidney function. Their baseline subject characteristics are presented in **Table 3.7**. All 16 participants completed both phases (placebo and experimental) of the experiment and attended both test days (Day 1 and Day 2) of each phase. The refined carbohydrate test meal and potassium phosphate pills administered in this experiment were well-tolerated by all participants, with no reports of gastrointestinal discomfort or adverse side effects. A Visual Analog Scale (VAS) organoleptic test, specifically designed for this study, was employed to evaluate the sensory characteristics of the test meal. Participants rated the sensory attributes of the test meal, including its smell, visual appeal, taste, aftertaste, and overall palatability (**Appendix J**). Responses were recorded on a 10-centimeter scale, with 0 cm indicating the least favorable rating and 10 cm indicating the most favorable rating. The results of the VAS assessment are presented in **Figure 3.10.**

Table 3.7. Baseline subject characteristics (Experiment 1).

N = 16	Males (n = 8)		Females (n = 8)	
Baseline characteristics	**Mean**	**SD**	**Mean**	**SD**
Age (years)	48.1	6.1	36.9	4.5
Weight (kg)	72.8	9.2	57.3	7.2
Height (cm)	175.6	8.6	161.6	6.1
BMI (kg/m^2)	23.5	1.6	21.9	1.2
Screening glucose (mg/dl) [a]	113.6	13.8	102.8	11.5
Screening ACR (mg/g Crea) [b]	7.3	3.6	7.1	3.2

SD, standard deviation; BMI: body mass index; ACR, albumin creatinine ratio; Crea, creatinine.
[a] Normal values: less than 180 mg/dl
[b] Normal values: less than 30 mg/g Crea

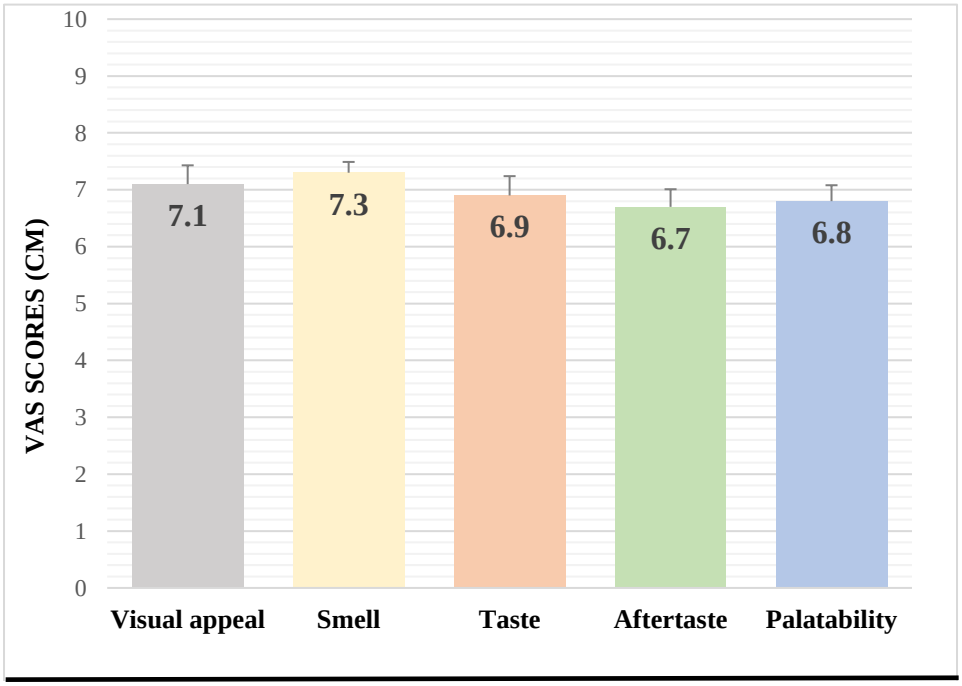


Figure 3.10. Organoleptic characteristics of refined carbohydrate test meal administered in Experiment 1, assessed using Visual Analogue Scale (VAS) questionnaire.

3.4.2 Postprandial glucose response

Glucose data were available for all 16 participants on both test days (Day 1 and Day 2). A repeated measures ANOVA, conducted using the General Linear Model (GLM), was employed to assess the effects of time (30, 60, 90, 120 minutes, etc.), treatment (placebo vs. phosphorus supplementation), day (Day 1 vs. Day 2), and their interactions on postprandial glucose response. Postprandial glucose response refers to changes in blood glucose levels (ΔBG) from baseline to subsequent time points. The analysis incorporated a within-subject factor for time and a between-subjects factor for treatment group. **Table 3.8** outlines the hypotheses tested in this statistical model, along with their corresponding results and interpretations.

When testing for the main effect of time, multivariate tests revealed a significant main effect of time on postprandial glucose response ($F(6, 55) = 96.165$, $p < 0.001$, $\eta^2 = 0.913$). Tests of within-subject effects confirmed that this effect remained significant across all sphericity corrections ($F(6, 55) = 139.314$, $p < 0.001$, $\eta^2 = 0.699$). These findings indicate substantial fluctuations in blood glucose levels over time, with a large effect size, as evidenced by the high partial eta squared values.

When testing for the effect of treatment, between-subject tests demonstrated a significant main effect of phosphorus supplementation on blood glucose levels across both intervention days (Day 1: $F(1, 30) = 24.335$, $p = 0.031$, $\eta^2 = 0.076$; Day 2: $F(1, 30) = 31.557$, $p = 0.006$, $\eta^2 = 0.082$), indicating that phosphorus significantly influenced overall postprandial blood glucose response. The placebo group experienced larger increases in blood glucose changes, with a mean change of 6.872 mg/dl (SE = 1.251, 95% CI: 6.369, 9.375), whereas the phosphorus group demonstrated smaller increases, with

a mean change of 2.951 mg/dl (SE = 1.251, 95% CI: 0.448, 5.454). The mean difference between both treatments was around 4 mg/dl and revealed statistical difference (SE = 1.770, 95% CI: 0.381, 7.461; $p = 0.031$).

Correspondingly, the interaction between time and treatment was statistically significant ($F(6, 55) = 4.995$, $p < 0.001$, $\eta^2 = 0.353$), suggesting that the effect of phosphorus supplementation on postprandial glucose response varied over time. This interaction remained significant across all within-subject corrections ($p < 0.001$, $\eta^2 = 0.108$), further confirming that phosphorus had a differential effect on blood glucose levels across time points. Pairwise comparisons revealed that at 30 minutes, ΔBG was higher in the placebo group (53.8 mg/dl, SE = 3.019, 95% CI: 47.80, 59.88) compared to the phosphorus group (37.4 mg/dl, SE = 3.041, 95% CI: 31.32, 43.40), and this difference persisted at 60 minutes, with the placebo group continuing to exhibit higher glucose values. However, as time progressed (from 90 minutes onwards), both groups showed a decline in glucose levels, though the phosphorus group maintained consistently lower overall blood glucose changes compared to the placebo group. Additionally, the phosphorus group demonstrated a faster return to baseline glucose levels by 60 minutes (ΔBG = -1.297 mg/dl, SE = 3.485), whereas the placebo group exhibited a slower return, reaching baseline by 90 minutes (ΔBG = -8.473 mg/dl, SE = 2.506). This pattern suggests a more efficient glucose clearance rate in the phosphorus group compared to the placebo group. The lower overall blood glucose changes observed in the phosphorus supplementation group, compared to the placebo group, indicate that phosphorus supplementation was associated with a more favorable and controlled glucose response across the measured time points, which was mediated by

both smaller post-meal fluctuations and a faster return to pre-meal baseline levels.

Finally, the effect of day was not statistically significant ($F(6, 55) = 1.817$, $p = 0.113$, $\eta^2 = 0.165$), indicating that postprandial glucose response pattern remained consistent across the two test days, with no evidence of a day effect or day-to-day variations. This is clearly illustrated in **Figure 3.11**, which shows that the mean postprandial glucose levels across both days followed a highly similar pattern, with minimal and statistically insignificant variations. Similarly, the three-way interaction between time, treatment, and day was not significant ($F(6, 55) = 0.600$, $p = 0.634$, $\eta^2 = 0.010$), further confirming that the observed postprandial glucose response following phosphorus supplementation remained consistent across both days, thereby reinforcing the reliability and reproducibility of the findings.

Table 3.8. Effects of time, treatment, day, and their interactions on postprandial glucose response.

	Hypothesis	***p*-value**	**Pairwise comparisons [a]**	**Indication**
Time	*Does postprandial glucose response change significantly over time?*	**<0.001***	Mean ΔBG across time points: – 30 min > 60 min – 30 min > 90 min – 30 min > 180 min	Postprandial glucose response was not uniform and followed a time-dependent pattern, with levels peaking at 30 minutes and gradually decreasing thereafter.
Treatment	*Does dietary phosphorus significantly affect postprandial glucose response?*	Day 1: **0.031*** Day 2: **0.006***	Mean ΔBG across treatments: – Phosphorus < placebo	Dietary phosphorus, when supplemented, reduced overall postprandial glucose response.
Day x Time	*Does postprandial glucose response differ between the two test days at various time points?*	0.128	Not applicable due to non-significant main effect.	Postprandial glucose levels were consistent at all time points, across Days 1 and 2, with no significant day effect observed.
Time x Treatment	*Does the effect of dietary phosphorus on postprandial glucose response vary over time?*	**<0.001***	Overall mean ΔBG peaks: – Placebo > phosphorus Glucose clearance: – Placebo < phosphorus	Dietary phosphorus resulted in lower overall blood glucose changes and faster return to baseline, compared to placebo.
Time x Treatment x Day	*Does the effect of dietary phosphorus on postprandial glucose response vary over time and between the two test days?*	0.634	Not applicable due to non-significant main effect.	Dietary phosphorus significantly impacted postprandial glucose response over time, and this effect remained consistent across both test days.

ΔBG, changes in blood glucose level from baseline.
Analysis performed via General Linear Model (Repeated Measures ANOVA).
[a] Pairwise comparisons were based on estimated marginal means.
* Indicates a statistically significant difference.
"<" symbol denotes a value less than or slower than the reference, and ">" denotes a value greater.

3.4.2.1 Postprandial glucose dynamics following test meal

Postprandial glucose dynamics, including magnitudes, durations, time to peak, postprandial glucose response, and incremental area under the curve (iAUC), are presented in **Table 3.9.** Baseline fasting glucose levels were similar between the phosphorus and placebo interventions on both days (Day 1: 98.2 ± 2.0 vs. 98.0 ± 2.7 mg/dl, p = 0.924; Day 2: 98.4 ± 2.5 vs. 98.9 ± 2.6 mg/dl, p = 0.760), indicating comparable starting points for both conditions. Following the glucose drink, peak glucose concentrations were significantly lower in the phosphorus group compared with placebo on both days, with mean differences of 17.6 mg/dl on Day 1 and 14.6 mg/dl on Day 2 (Day 1: 136.5 ± 4.9 vs. 154.1 ± 5.2 mg/dl, p < 0.0001; Day 2: 134.8 ± 3.9 vs. 149.4 ± 4.5 mg/dl, p < 0.0001) (**Table 3.9**). The greatest effect was observed at 30 minutes, where the placebo group demonstrated a substantial increase in blood glucose levels by 56.1 mg/dl (SE = 3.0), corresponding to a 57% rise from baseline, whereas the phosphorus group exhibited a comparatively lower increase of 38.8 mg/dl (SE = 3.0), representing a 40% rise from baseline. The mean difference between groups at this time point was 17.3 mg/dl (p < 0.0001). Similarly, at 60 minutes, the placebo group experienced a rise in blood glucose from baseline levels by 21.2 mg/dl (SE = 3.9) on Day 1, while the phosphorus group returned close to pre-prandial levels, with a ΔBG of 2.2 mg/dl (SE = 3.1). The mean difference between the two groups remained consistent at 19 mg/dl (SE = 2.5), and this difference was highly significant (p < 0.0001), indicating a strong effect of phosphorus supplementation on early postprandial glycemic response.

Similarly, the peak glucose increment, calculated as the difference between peak and baseline glucose levels, was markedly attenuated with phosphorus relative to placebo on both days, with mean differences of 17.8 mg/dl on

Day 1 and 15.2 mg/dl on Day 2 (Day 1: 38.3 ± 4.6 vs. 56.1 ± 4.4 mg/dl, $p < 0.0001$; Day 2: 36.4 ± 3.8 vs. 51.6 ± 4.3 mg/dl, $p < 0.0001$). The time to reach peak glucose however, did not differ between interventions (Day 1: 26.3 ± 2.2 vs. 26.3 ± 1.8 min, $p = 1.000$; Day 2: 23.8 ± 1.7 vs. 24.1 ± 1.7 min, $p = 0.896$), suggesting that phosphorus supplementation did not alter the rate of glucose appearance. Beyond 90 minutes, differences between groups were no longer statistically significant at individual time points (90–180 min, all $p > 0.3$), though the phosphorus group consistently exhibited lower blood glucose values compared to placebo. Integrated measures further highlighted these differences: the incremental area under the curve (iAUC) for postprandial glucose demonstrated consistently lower positive iAUC values following phosphorus supplementation compared with the placebo condition across both study days. The positive iAUC, representing the total glucose exposure above baseline, was significantly lower in the phosphorus-supplemented condition relative to placebo on both Day 1 (18,355 ± 964 mg·min/dL vs. 19,332 ± 1,071 mg·min/dL, $p < 0.0001$) and Day 2 (18,284 ± 953 mg·min/dL vs. 18,934 ± 995 mg·min/dL, $p < 0.0001$). This pattern indicates a consistent improvement in postprandial glucose regulation with phosphorus supplementation.

The negative iAUC, reflecting the area below baseline and indicative of glucose decline during the late postprandial phase, did not differ significantly between the phosphorus and placebo conditions on either day (Day 1: –787 ± 126 mg·min/dL vs. –700 ± 125 mg·min/dL, $p = 0.950$; Day 2: –830 ± 131 mg·min/dL vs. –723 ± 133 mg·min/dL, $p = 0.323$). These findings suggest that while phosphorus supplementation effectively reduced the magnitude of postprandial glucose excursions, it did not substantially alter the degree of glucose decline below fasting levels during recovery.

Overall, these findings indicate that phosphorus supplementation significantly reduced postprandial glucose excursions in both magnitude and duration during the early phase of the glucose response, without altering the initial fasting glucose concentration or the time to peak glucose. **Figures 3.11** and **3.12** illustrate the mean blood glucose concentrations and the mean ΔBG changes, respectively, across the treatment groups on both intervention Days 1 and 2.

Table 3.9. Postprandial glucose dynamics following test meal: concentrations, peak time, magnitude, and duration over 180 minutes on Days 1 and 2.

	Phosphorus	Placebo	*p*-value
Baseline glucose (mg/dl)			
Day 1	98.2 ± 2.0	98.0 ± 2.7	0.924
Day 2	98.4 ± 2.5	98.9 ± 2.6	0.760
Peak glucose (mg/dl)			
Day 1	136.5 ± 4.9	154.1 ± 5.2	**<0.0001***
Day 2	134.8 ± 3.9	149.4 ± 4.5	**<0.0001***
Time to peak (min)			
Day 1	26.3 ± 2.2	26.3 ± 1.8	1.000
Day 2	23.8 ± 1.7	24.1 ± 1.7	0.896
Peak glucose increment (mg/dl)			
Day 1	38.3 ± 4.6	56.1 ± 4.4	**<0.0001***
Day 2	36.4 ± 3.8	51.6 ± 4.3	**<0.0001***
ΔBG at 30 min (mg/dl)			
Day 1	38.8 ± 3.0	56.1 ± 3.0	**<0.0001***
Day 2	36.4 ± 2.8	50.5 ± 2.9	**<0.0001***
ΔBG at 60 min (mg/dl)			
Day 1	2.2 ± 3.1	21.2 ± 3.9	**<0.0001***
Day 2	-4.8 ± 2.8	7.2 ± 2.9	**<0.0001***
ΔBG at 90 min (mg/dl)			
Day 1	-8.5 ± 2.5	-8.7 ± 2.4	0.520
Day 2	-5.6 ± 1.8	-9.3 ± 2.1	0.317
ΔBG at 120 min (mg/dl)			
Day 1	-5.0 ± 1.9	-4.9 ± 2.2	0.342
Day 2	-3.6 ± 2.1	-8.8 ± 2.9	0.449
ΔBG at 150 min (mg/dl)			
Day 1	-5.2 ± 1.7	-5.6 ± 1.6	0.630
Day 2	-1.5 ± 0.3	-4.3 ± 1.3	0.438
ΔBG at 180 min (mg/dl)			
Day 1	2.7 ± 2.1	-2.7 ± 1.7	0.778
Day 2	-3.6 ± 0.8	-1.0 ± 0.7	0.651
Positive iAUC (mg·min/dl)			
Day 1	18,355 ± 964	19,332 ± 1,071	**<0.0001***
Day 2	18,284 ± 953	18,934 ± 995	**<0.0001***
Negative iAUC (mg·min/dl)			
Day 1	-787 ± 126	-700 ± 125	0.950
Day 2	-830 ± 131	-723 ± 133	0.323

ΔBG, changes in blood glucose levels; iAUC, incremental area under the curve; min, minutes.
Data presented as mean ± standard error of the mean (SEM). A 2-tailed paired t-test was performed between groups. *Indicates statistical significance ($p < 0.05$).

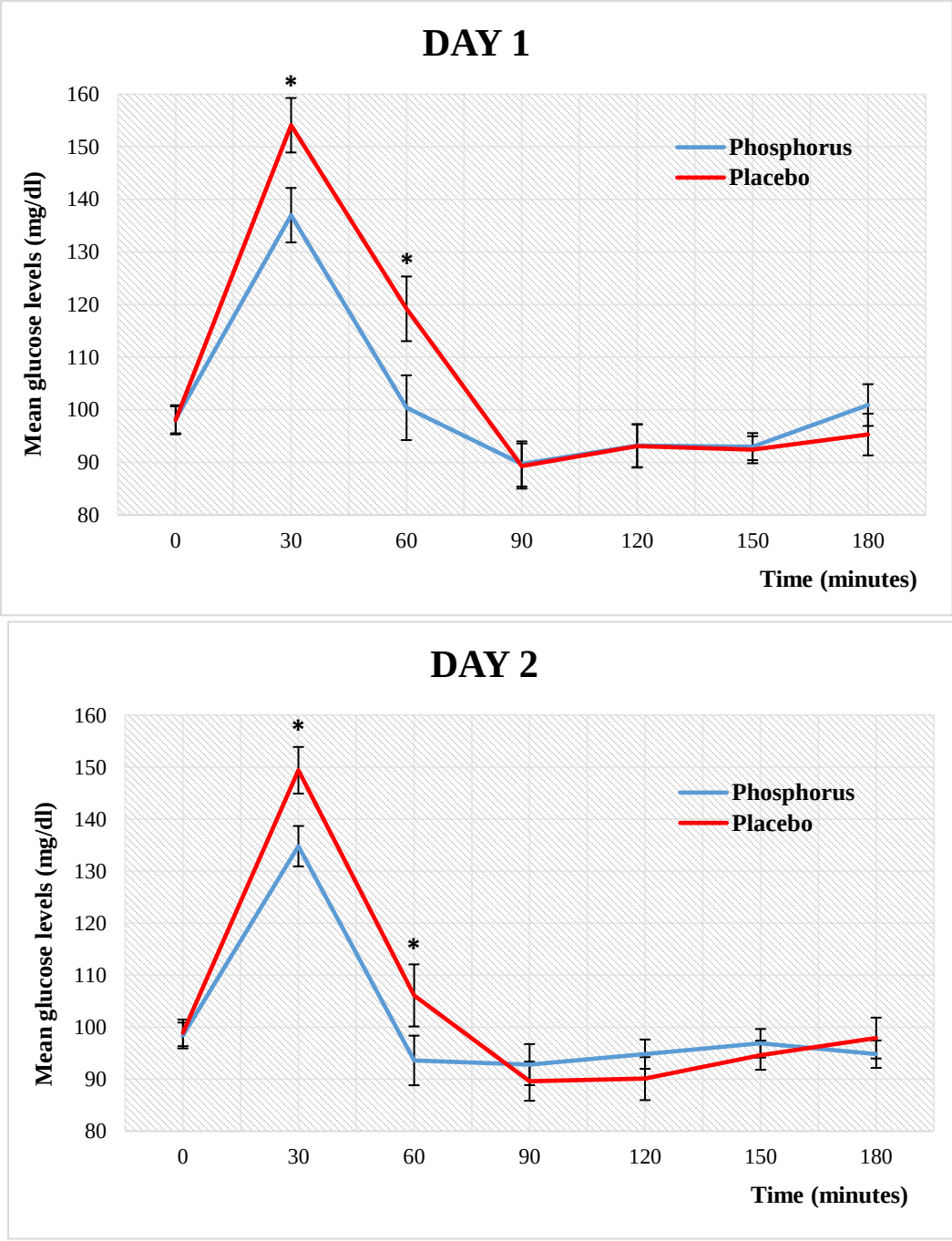


Figure 3.11. Time course of postprandial blood glucose concentrations in response to the test meal across intervention Days 1 and 2.
Vertical bars indicate standard error of the mean (SEM).
*Indicates a statistically significant difference between both groups ($p < 0.05$).

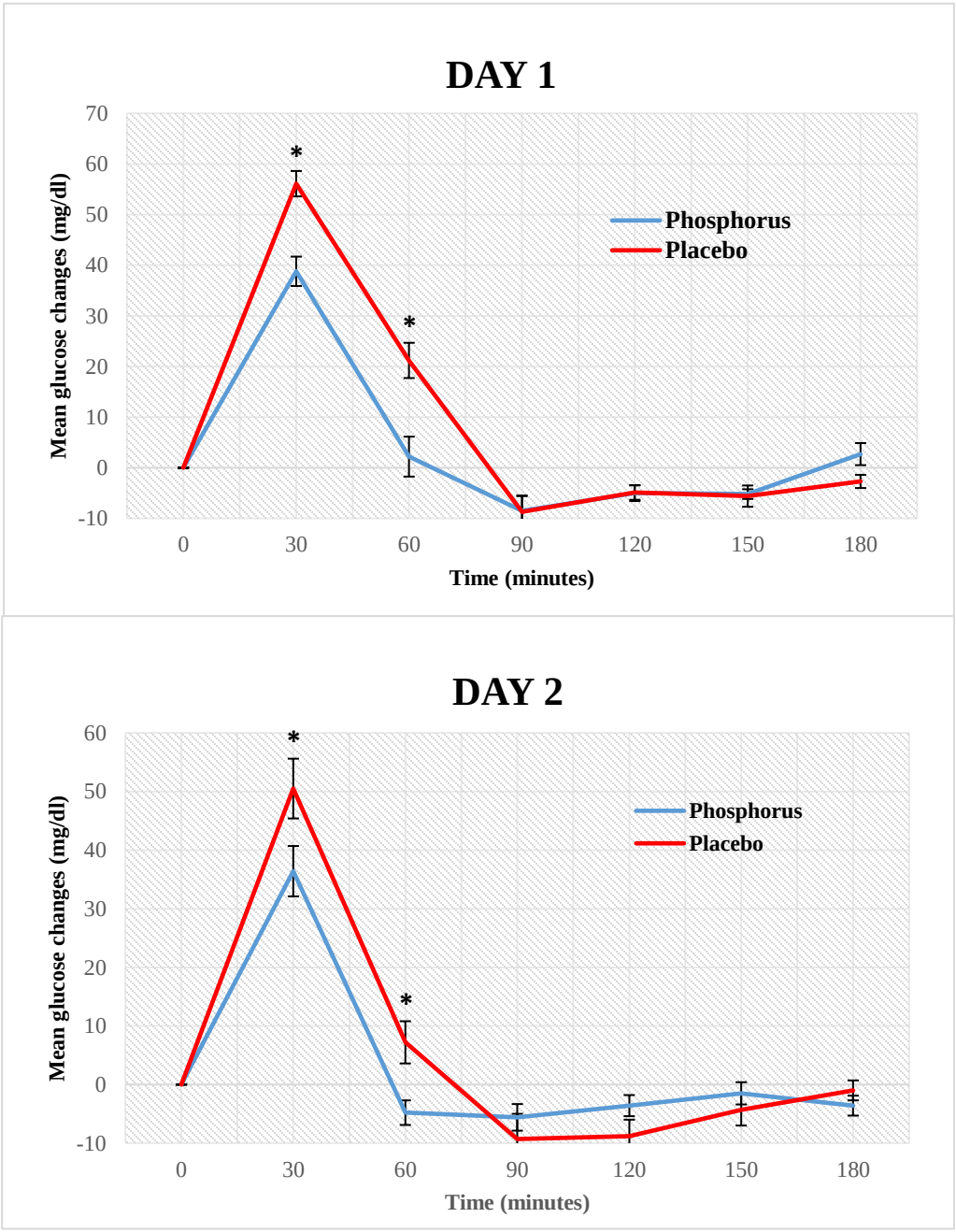


Figure 3.12. Time course of postprandial changes in blood glucose (ΔBG) in response to the test meal across intervention Days 1 and 2.

Vertical bars indicate standard error of the mean (SEM).

*Indicates a statistically significant difference between both groups ($p < 0.05$).

3.4.3 Postprandial core body temperature

A multivariate repeated measures ANOVA was conducted to examine the effects of time, day, treatment, and their interactions on postprandial CBT. The analysis revealed no significant main effect of time ($F(6, 55) = 2.019$, $p = 0.079$, $\eta^2 = 0.180$), indicating that postprandial CBT did not vary significantly over time.

Similarly, no significant effect of treatment group was observed ($F(1, 60) = 2.622$, $p = 0.111$, $\eta^2 = 0.042$). The placebo group had a mean CBT of 0.021°C (SE = 0.034), and the phosphorus supplementation group had a mean of 0.056°C (SE = 0.034), with no significant difference between the groups (mean difference = -0.077°C, $p = 0.111$).

Furthermore, when testing for the effect of time and treatment, postprandial CBT variations did not significantly differ between the two treatment groups ($F(6, 55) = 1.210$, $p = 0.315$, $\eta^2 = 0.117$), indicating no significant difference in CBT changes between the placebo and phosphorus supplementation groups across time points.

The day factor also did not significantly influence CBT changes ($F(1, 60) = 0.555$, $p = 0.459$, $\eta^2 = 0.009$), and there were no significant interactions between day and treatment or day and time. Finally, the three-way interaction between time, treatment group, and day was not significant ($F(6, 55) = 1.235$, $p = 0.303$, $\eta^2 = 0.119$).

In a follow-up univariate analysis, postprandial core body temperature (CBT) was examined across intervention days. At baseline, mean CBT values were comparable between the phosphorus and placebo conditions on both intervention days, indicating similar starting thermic states prior to meal ingestion (Day 1: 37.07 ± 0.05 °C vs. 37.10 ± 0.07 °C, $p = 0.524$; Day 2: 37.16 ± 0.06 °C vs. 37.07 ± 0.05 °C, $p = 0.071$).

Following the glucose drink, minor fluctuations in CBT were observed over the 180-minute postprandial period in both conditions, with no significant differences at any time point **(Table 3.10)**. **Figures 3.13** and **3.14** provide a visual representation of the mean CBT and mean ΔCBT over time for both treatment groups. On both days, CBT tended to rise slightly within the first 30–60 minutes, reaching small positive changes in the phosphorus condition (maximum ΔCBT ≈ +0.07–0.08 °C) compared with near-zero or slightly negative changes under placebo. However, these increases did not reach statistical significance (all $p > 0.05$).

Beyond 90 minutes, CBT changes stabilized or gradually declined toward baseline in both groups, showing minimal variation up to 180 minutes. The absence of significant differences across conditions indicates that phosphorus supplementation did not meaningfully alter postprandial thermogenic or heat-dissipation responses under the current experimental conditions.

Table 3.10. Mean postprandial core body temperature variations over 180 minutes on Days 1 and 2.

	Phosphorus	Placebo	*p*-value
CBT at baseline (°C)			
Day 1	37.07 ± 0.05	37.1 ± 0.07	0.524
Day 2	37.16 ± 0.06	37.07 ± 0.05	0.071
ΔCBT at 30 min (°C)			
Day 1	0.07 ± 0.04	0.01 ± 0.05	0.134
Day 2	0.08 ± 0.06	0.02 ± 0.04	0.085
ΔCBT at 60 min (°C)			
Day 1	0.08 ± 0.05	-0.06 ± 0.06	0.386
Day 2	0.05 ± 0.09	0.02 ± 0.04	0.215
ΔCBT at 90 min (°C)			
Day 1	0.06 ± 0.05	-0.04 ± 0.04	0.095
Day 2	0.08 ± 0.09	0.04 ± 0.05	0.783
ΔCBT at 120 min (°C)			
Day 1	0.03 ± 0.05	-0.04 ± 0.04	0.733
Day 2	0.06 ± 0.09	0.03 ± 0.05	0.456
ΔCBT at 150 min (°C)			
Day 1	-0.01 ± 0.06	-0.06 ± 0.05	0.793
Day 2	0.02 ± 0.06	0.04 ± 0.06	0.712
ΔCBT at 180 min (°C)			
Day 1	0.01 ± 0.05	-0.06 ± 0.05	0.922
Day 2	0.03 ± 0.07	0.03 ± 0.06	0.499

ΔCBT, changes in core body temperature; °C, degree Celsius.
Data presented as mean ± standard error of the mean (SEM). A 2-tailed paired t-test was performed between groups.

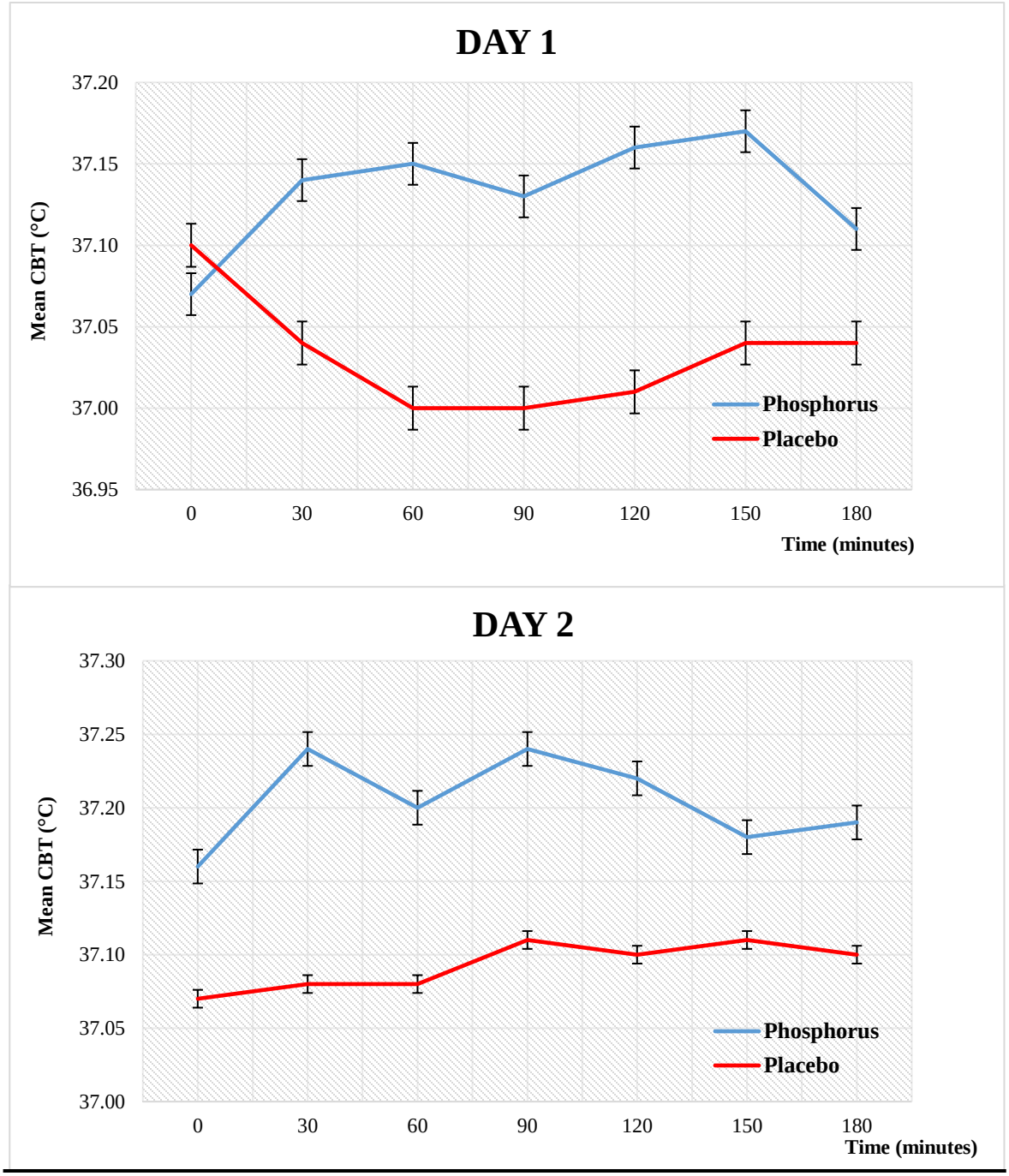


Figure 3.13. Time course of postprandial core body temperature in response to the test meal across intervention Days 1 and 2.
Vertical bars indicate standard error of the mean (SEM).

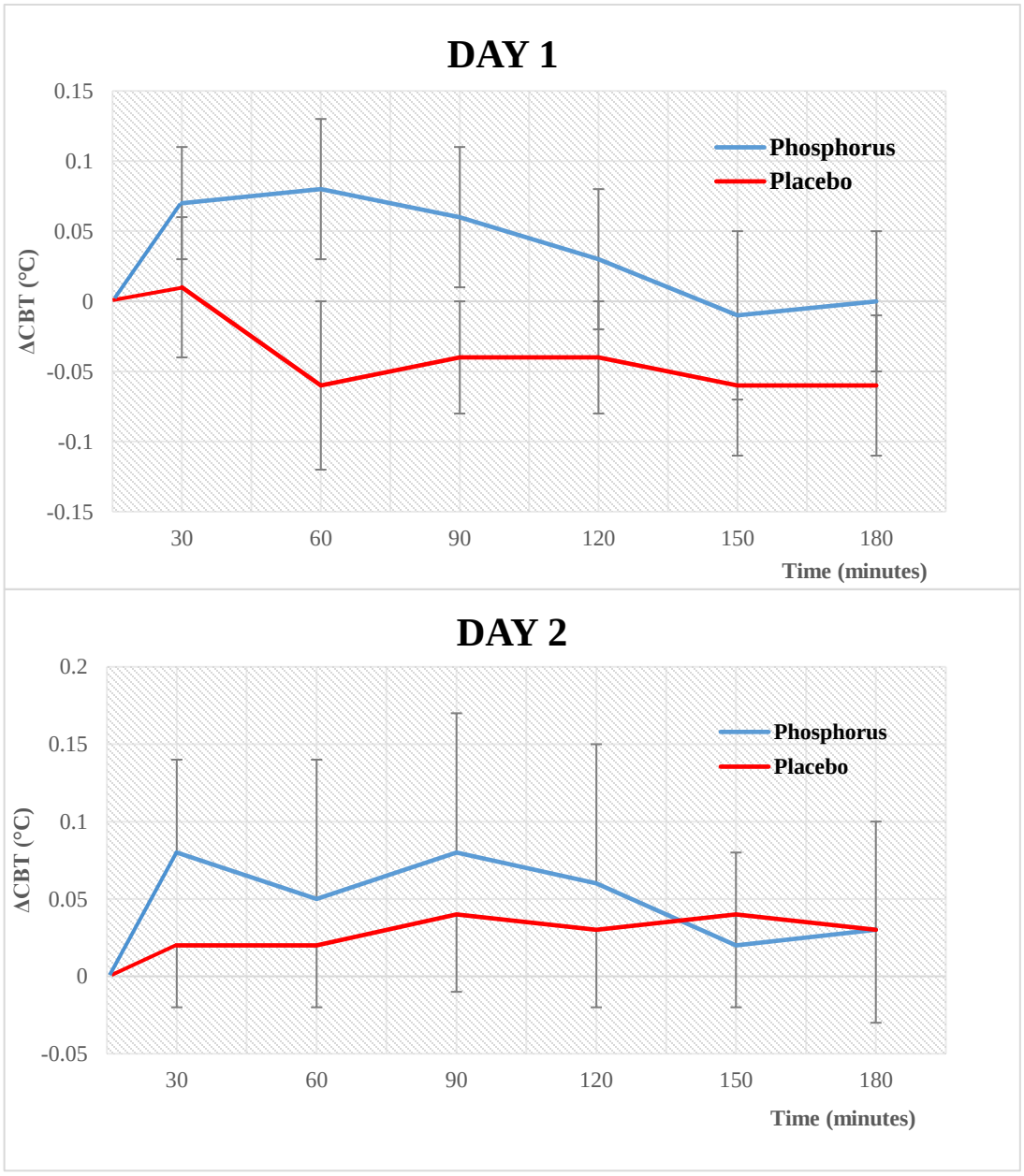


Figure 3.14. Time course of postprandial changes in core body temperature (ΔCBT) in response to the test meal across intervention Days 1 and 2.
Vertical bars indicate standard error of the mean (SEM).

3.4.4 Appetite and food preference assessments

Subjective appetite and food preference assessments were conducted using the visual analog scale (VAS). Measurements were recorded at baseline, followed by a series of assessments at 15-minute intervals over a two-hour period post meal ingestion. All 16 participants completed the questionnaire on both days of each intervention.

The VAS questionnaire assessed four key appetite sensations: hunger, satiety, fullness, and prospective food consumption (PFC), with the corresponding grading system detailed below:

- Hunger: lower scores indicated *not hungry at all*, while higher scores reflected *extreme hunger*.
- Satiety: the lowest score corresponded to *feeling completely empty*, whereas the highest indicated an *inability to eat more.*
- Fullness: a lower score represented *not full at all*, while a higher score denoted *feeling totally full*.
- PFC: the lowest score signified *no desire to eat*, while the highest indicated a *capacity to eat a lot*.

A three-way repeated measures ANOVA was conducted to evaluate the effects of time, treatment, and their interaction on subjective appetite sensations (hunger, satiety, fullness, and prospective food consumption (PFC)) as well as on specific food preferences (sweet, salty, fatty, and savory) across the two intervention days (**Table 3.11**). Across both days, time had a highly significant effect ($p < 0.001$) on all four appetite variables, indicating that subjective sensations of hunger, satiety, fullness, and PFC changed meaningfully throughout the postprandial period. This reflects the expected physiological pattern following meal ingestion, with hunger and PFC decreasing while satiety and fullness increased over time.

Similarly, the time × treatment interactions were highly significant for all appetite measures on both days (all $p < 0.05$), indicating that phosphorus supplementation consistently influenced postprandial appetite dynamics compared with placebo. Significant differences emerged from the early postprandial period onward, with hunger and satiety reaching significance from 30 minutes, and fullness and PFC from 45 minutes post-meal, as illustrated in **Figures 3.15** and **3.16**. Participants receiving phosphorus reported lower hunger and PFC scores and higher satiety and fullness scores relative to placebo, reflecting an appetite-suppressive effect of phosphorus that persisted throughout the postprandial phase. The reproducibility of these interactions across both study days underscores the robust effect of phosphorus on modulating appetite responses over time.

Regarding food preferences, postprandial ratings exhibited significant time effects across most categories, indicating that participants' desire for sweet, salty, fatty, and savory foods changed substantially over the postprandial period. Specifically, sweet and fatty preferences decreased markedly over time on both days (Day 1 sweet: $p = 0.000$, fatty: $p = 0.000$; Day 2 sweet: $p = 0.001$, fatty: $p = 0.023$), while salty and savory preferences also showed significant temporal variations on Day 1 (salty: $p = 0.002$; savory: $p = 0.000$) and to a lesser extent on Day 2 (salty: $p = 0.009$; savory: $p = <0.001$). In contrast, treatment effects were generally minimal, with only salty preference on Day 1 showing a modest effect of phosphorus versus placebo ($p = 0.023$), and no other categories demonstrating significant differences. Likewise, the time × treatment interactions were not significant for any food preference variable on either day (all $p > 0.277$), indicating that the temporal evolution of food preference ratings was not meaningfully influenced by phosphorus supplementation.

Taken together, these results suggest that while phosphorus robustly modulated overall appetite sensations, reducing hunger and PFC while enhancing satiety and fullness, the effects on specific food preferences were limited. Postprandial declines in the desire for sweet, salty, fatty, and savory foods occurred predominantly as a function of time, independent of treatment, highlighting that the primary impact of phosphorus was on general appetite control rather than targeted macronutrient cravings.

Table 3.11. Repeated measures ANOVA for postprandial subjective appetite ratings and food preferences over 120 minutes across two intervention days.

Outcome variables	Time (p value)	Treatment (p value)	Time x treatment (p value)
Hunger			
Day 1	**0.000**[a]	**0.000**[a]	**0.041**[c]
Day 2	**0.000**[a]	**0.000**[a]	**0.007**[b]
Satiety			
Day 1	**0.000**[a]	**0.000**[a]	**0.000**[a]
Day 2	**0.000**[a]	**0.000**[a]	**0.000**[a]
Fullness			
Day 1	**0.000**[a]	**0.000**[a]	**0.000**[a]
Day 2	**0.000**[a]	**0.000**[a]	**0.000**[a]
PFC			
Day 1	**0.000**[a]	**0.000**[a]	**0.000**[a]
Day 2	**0.000**[a]	**0.000**[a]	**0.002**[b]
Sweet			
Day 1	**0.000**[a]	0.696	0.787
Day 2	**0.001**[b]	0.837	0.870
Salty			
Day 1	**0.002**[b]	**0.023**[c]	0.646
Day 2	**0.009**[b]	0.396	0.343
Fatty			
Day 1	**0.000**[a]	0.603	0.489
Day 2	**0.023**[c]	0.912	0.277
Savory			
Day 1	**0.000**[a]	0.824	0.885
Day 2	**0.000**[a]	0.093	0.775

PFC, prospective food consumption.
[a] Indicates statistical significance ($p < 0.001$) ,[b] ($p < 0.01$), and [c] ($p < 0.05$), based on repeated measures ANOVA.

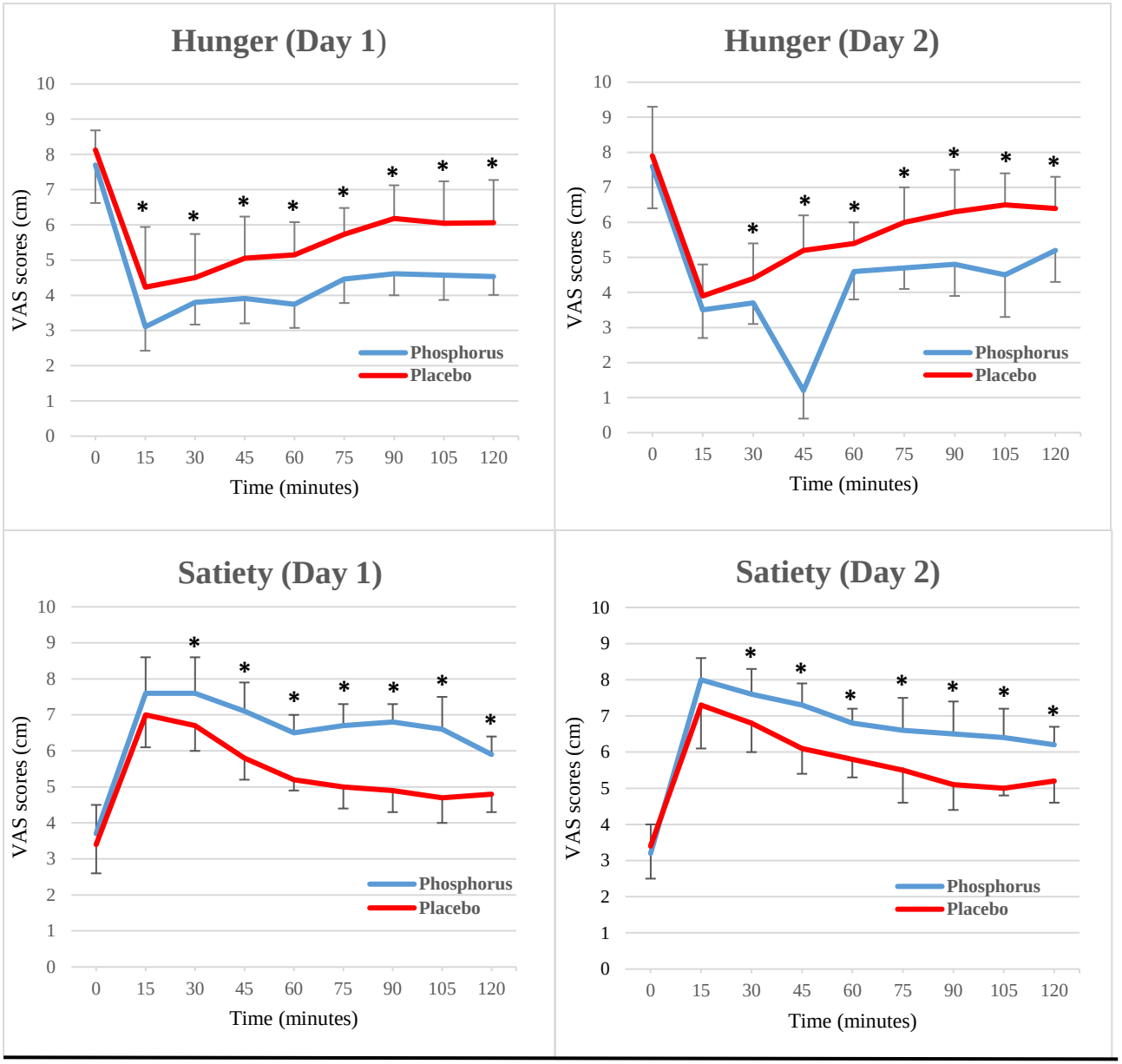


Figure 3.15. Changes in subjective appetite ratings (hunger and satiety) from baseline to 120 minutes following test meal ingestion.

Vertical bars indicate standard deviation.

*Indicates a statistically significant difference between both groups ($p < 0.05$).

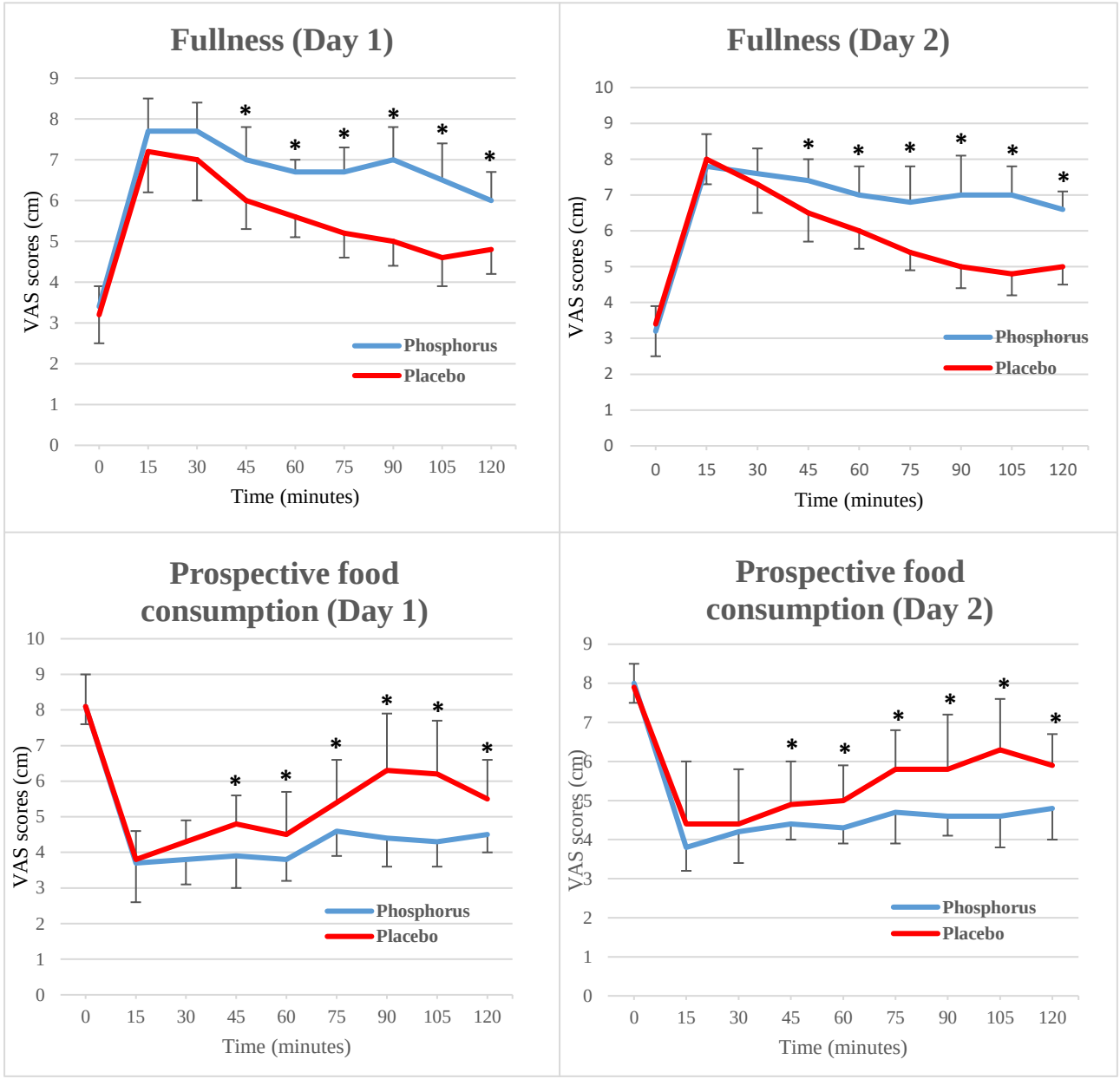


Figure 3.16. Changes in subjective appetite ratings (fullness and prospective food consumption) from baseline to 120 minutes following test meal ingestion.

Vertical bars indicate standard deviation.

3.5 Results (Experiment 2)

3.5.1 Baseline characteristics

This experiment included a total of 16 participants (8 males and 8 females), all of whom met the established inclusion criteria. Participants had normal BMI values, glucose levels, and kidney function. Their baseline subject characteristics are presented in **Table 3.12**. All 16 participants completed both phases (placebo and experimental) of the experiment and attended both test days (Day 1 and Day 2) of each phase. The sevelamer carbonate tablets were well-tolerated by all participants, with no reports of gastrointestinal discomfort or adverse side effects.

Table 3.12. Baseline subject characteristics (Experiment 2).

N = 16	**Males (n = 8)**		**Females (n = 8)**	
Baseline characteristics	**Mean**	**SD**	**Mean**	**SD**
Age (years)	23.4	7.9	22.9	4.3
Weight (kg)	70.1	3.5	50.1	11.7
Height (cm)	175.2	3.9	161.2	3.1
BMI (kg/m^2)	22.9	1.9	21.0	0.8
Screening glucose (mg/dl) [a]	94.9	6.8	93.8	3.9
Screening ACR (mg/g Crea) [b]	4.6	2.4	5.08	1.1

SD, standard deviation; BMI: body mass index; ACR, albumin creatinine ratio; Crea, creatinine.
[a] Normal values: less than 180 mg/dl
[b] Normal values: less than 30 mg/g Crea

3.5.2 Postprandial glucose response

Due to a technical malfunction of the DEXCOM continuous glucose monitoring device, glucose data from one participant were unavailable on Day 2. Consequently, Day 2 analyses were based on 15 participants, whereas Day 1 analyses included all 16 participants.

A repeated measures ANOVA, implemented via the General Linear Model (GLM), was performed to evaluate the effects of time (30, 60, 90, 120 minutes, etc.), treatment (placebo vs. phosphorus supplementation or placebo vs. chelator), day (Day 1 vs. Day 2), and their interactions on postprandial glucose response. The model included time as a within-subject factor and treatment as a between-subjects factor. **Table 3.13** presents the specific hypotheses tested within this model, along with the corresponding results and interpretations.

When testing for the effect of time, changes in blood glucose levels (ΔBG) varied significantly across time points, indicating a time-dependent pattern in postprandial glucose response ($F (6, 54) = 6.358$, $p < 0.001$, $\eta^2 = 0.413$) (**Table 3.13**). Tests of within-subject contrasts (time trends) were also conducted. Results revealed a significant linear trend ($F (1, 61) = 12.888$, $p = 0.001$), indicating a consistent change in blood glucose levels over time. Additionally, a significant quadratic trend ($F (1, 61) = 26.400$, $p < 0.001$) was observed, suggesting a rise-and-fall pattern in glucose response. The largest increase in blood glucose levels was observed at 30 minutes, with a mean of 6.132 mg/dl (SE = 1.044, 95% CI: 4.044, 8.220), while a significant decrease in blood glucose levels compared to earlier peaks occurred at 180 minutes, with a mean of -2.750 mg/dl (SE = 1.150, 95% CI: -5.051, -0.449). Overall, blood glucose levels exhibited a significant post-meal rise at 30 minutes, followed by a gradual return to baseline by 180 minutes.

Similarly, testing the effect of treatment revealed that dairy-derived phosphorus intake significantly reduces overall postprandial glucose response on both intervention days (Day 1: F (1, 30) = 7.854, $p < 0.009$, $\eta^2 = 0.207$; Day 2: F (1, 30) = 22.5, $p < 0.001$, $\eta^2 = 0.433$) (**Table 3.13**). In contrast, the chelator group experienced larger increases in blood glucose changes, with a mean change of 4.874 mg/dl (SE = 0.810, 95% CI: 3.254, 6.493), whereas the placebo group demonstrated smaller increases or even decreases at some points, with a mean change of -1.071 mg/dl (SE = 0.796, 95% CI: -2.665, 0.522. The mean difference between both treatments was around 6 mg/dl and was statistically significant (SE = 1.136, 95% CI: 3.673, 8.217, $p < 0.001$). The model also revealed that postprandial glucose levels were not statistically different across Days 1 and 2 (F (6, 54) = 0.362, $p = 0.899$). This effect was consistent within each intervention group (placebo and chelator), indicating the absence of a day effect or day-to-day variations.

The analysis of the interaction between time and treatment revealed significant patterns in postprandial glucose response between the placebo and chelator groups over time (F (1, 59) = 6.052, $p = 0.017$, $\eta^2 = 0.372$), as outlined in **Table 3.13**. The latter exhibited higher peaks in blood glucose changes across all time points compared to the placebo group, with significant differences observed at 30, 60, 90, and 150 minutes. At 30 minutes, the chelator group reached a mean ΔBG peak of 10.765 mg/dl (SE = 1.488, 95% CI: 7.787, 13.742), whereas the placebo group showed a mean of 1.500 mg/dl (SE = 1.464, 95% CI: -1.429, 4.429). Similarly, at 60 minutes, the chelator group demonstrated a mean peak of 7.769 mg/dl (SE = 1.838, 95% CI: 4.091, 11.447), while the placebo group had a mean of 0.094 mg/dl (SE = 1.808, 95% CI: -3.524, 3.712). At 90 minutes, the

chelator group displayed a mean ΔBG peak of 7.360 mg/dl (SE = 1.710, 95% CI: 3.939, 10.782), in contrast to the placebo group's mean of 0.219 (SE = 1.682, 95% CI: -3.147, 3.584). Finally, at 150 minutes, the chelator group exhibited a mean ΔBG peak of 3.829 mg/dl (SE = 1.473, 95% CI: 0.881, 6.778), compared to the placebo group -3.344 mg/dl (SE = 1.449, 95% CI: -6.244, -0.443). Furthermore, the chelator group demonstrated a slower return to baseline, taking up to 180 minutes (ΔBG = -0.406 mg/dl, SE = 1.640), whereas the placebo group exhibited a faster return to baseline, starting at 60 minutes (ΔBG = 0.094 mg/dl, SD = 1.808). This resulted in a difference of nearly 120 minutes in glucose clearance time between the two groups, with the placebo group demonstrating a more efficient glucose clearance from the bloodstream. The interaction between time and treatment group also exhibited a quadratic trend ($F(1, 61) = 6.240$, $p = 0.015$), indicating that the rise-and-fall pattern differed between both groups. Overall, these findings suggest that blood glucose levels followed a significant rise-and-fall trajectory, with the placebo group displaying a more moderated response. The results indicate that dairy-derived phosphorus mitigates glucose fluctuations by reducing the magnitude of glucose spikes and accelerating the return to baseline, thereby stabilizing the postprandial glucose response curve.

Finally, the combined effect of time, treatment, and day did not yield statistical significance ($F(6, 54) = 1.613$, $p = 0.161$), indicating the absence of a three-way interaction (**Table 3.13**).

Put together, these findings suggest that dairy-derived phosphorus significantly improved postprandial glucose levels over time, and this effect remained consistent across both test days.

Table 3.13. Effects of time, treatment, day, and their interactions on postprandial glucose response.

	Hypothesis	*p*-value	Pairwise comparisons [a]	Indication
Time	*Does postprandial glucose response change significantly over time?*	**<0.001***	Mean ΔBG across time points: – 30 min > 60 min – 30 min > 90 min – 30 min > 180 min	Postprandial glucose response was not uniform and followed a time-dependent pattern, with levels peaking at 30 minutes and gradually decreasing thereafter.
Treatment	*Does dairy phosphorus chelation significantly affect postprandial glucose response?*	Day 1: **0.009*** Day 2: **<0.001***	Mean ΔBG across treatments: – Chelator > placebo	Phosphorus chelation increased overall postprandial glucose response.
Day x Time	*Does postprandial glucose response differ between the two test days at various time points?*	0.899	Not applicable due to non-significant main effect.	Postprandial glucose levels were consistent at all time points, across Days 1 and 2, with no significant day effect observed.
Time x Treatment	*Does the effect of dairy phosphorus chelation on postprandial glucose response vary over time?*	**0.017***	Overall mean ΔBG peaks: – Chelator > placebo Glucose clearance: – Chelator < placebo	Phosphorus chelation resulted in higher overall blood glucose changes and slower return to baseline levels, compared to placebo.
Time x Treatment x Day	*Does the effect of dairy phosphorus chelation on postprandial glucose response vary over time and between the two test days?*	0.161	Not applicable due to non-significant main effect.	Phosphorus chelation significantly impacted postprandial glucose response over time, and this effect remained consistent across both test days.

ΔBG, changes in blood glucose level from baseline.
Analysis performed via General Linear Model (Repeated Measures ANOVA).
[a] Pairwise comparisons were based on estimated marginal means.
* Indicates a statistically significant difference.
"<" symbol denotes a value less than or slower than the reference, and ">" denotes a value greater.

3.5.2.1 Postprandial glucose dynamics following test meal

Postprandial glucose dynamics, including magnitudes, durations, time to peak, postprandial glucose response, and incremental area under the curve (iAUC), are presented in **Table 3.14**. The postprandial glucose response demonstrated distinct differences between the placebo and chelator treatments across both intervention days. Baseline glucose concentrations were comparable between groups, with values slightly higher in the placebo condition (Day 1: 110.0 ± 2.3 mg/dL vs. 106.1 ± 3.6 mg/dL; p = 0.229; Day 2: 108.7 ± 2.8 mg/dL vs. 104.0 ± 3.2 mg/dL; p = 0.093), indicating similar baseline glycemic states. However, marked differences emerged in the postprandial phase.

The time to reach peak glucose concentration was significantly shorter in the chelator group on both days (Day 1: 67.5 ± 8.9 min vs. 106.9 ± 12.2 min, p = 0.021; Day 2: 72.0 ± 11.6 min vs. 98.0 ± 13.2 min, p = 0.041), representing an approximate 37–45% faster rise in glucose following milk ingestion with the phosphorus chelator. Correspondingly, the peak glucose increment from baseline was significantly greater in the chelator condition (Day 1: +17.0 ± 2.2 mg/dL vs. +10.0 ± 1.6 mg/dL, p = 0.006; Day 2: +16.4 ± 1.9 mg/dL vs. +7.5 ± 2.2 mg/dL, p < 0.001), indicating an approximately 70–120% higher postprandial glucose excursion compared with the placebo.

The time-course analysis of incremental glucose changes further supported this pattern. At 30 minutes post-meal, the chelator group exhibited a sharp and significant rise in glucose levels compared with placebo (Day 1: +12.1 ± 2.0 mg/dL vs. +1.0 ± 1.2 mg/dL, p < 0.001; Day 2: +9.5 ± 2.6 mg/dL vs.

+1.7 ± 2.4 mg/dL, p = 0.002), corresponding to nearly a tenfold greater early postprandial increase.

The greatest treatment effect was observed at 30 minutes, where the difference between groups was maximal, reflecting the most pronounced impairment in glucose regulation when phosphorus availability was reduced. This elevated response persisted through 60, 90, and 120 minutes, with significantly higher glucose levels in the chelator condition at nearly all time points ($p < 0.05$), particularly evident at 60, 90, and 120 minutes on both days. By 150 minutes, the placebo group's glucose levels had declined below baseline (−6.8 ± 2.0 mg/dL on Day 2), while the chelator group remained elevated (+6.5 ± 2.0 mg/dL, p = 0.001), suggesting a delayed return to baseline and prolonged glycemic exposure. At 180 minutes, this difference persisted on Day 2 (p = 0.017), further highlighting the sustained elevation of glucose levels in the absence of available phosphorus.

Analysis of the incremental area under the curve (iAUC) revealed marked differences in postprandial glucose dynamics between the dairy milk and phosphorus-chelator conditions. The positive iAUC, representing the magnitude of the glucose rise above baseline, was significantly lower in the dairy milk condition compared with the chelator condition on both study days (Day 1: 522.2 ± 107.8 mg·min/dL vs. 1300.3 ± 210.6 mg·min/dL, p = 0.004; Day 2: 463.1 ± 134.8 mg·min/dL vs. 1260.0 ± 143.1 mg·min/dL, $p < 0.001$). This consistent pattern across both test days indicates that the presence of bioavailable dairy-derived phosphorus substantially reduced total postprandial glucose exposure.

In contrast, the negative iAUC, which reflects the area below baseline and denotes the extent of glucose undershoot during late postprandial recovery, did not differ significantly between treatments on Day 1 (–487.5 ± 68.6

mg·min/dL vs. –326.3 ± 85.1 mg·min/dL, p = 0.155). However, by Day 2, a significant difference emerged (–795.0 ± 142.0 mg·min/dL vs. –175.0 ± 74.5 mg·min/dL, p = 0.004), with a more pronounced negative area observed following dairy milk ingestion. Together, these findings show that while the chelator condition was associated with higher and more prolonged glucose elevations, the dairy milk condition promoted an earlier return of glucose to baseline and a greater postprandial decline below fasting levels, particularly evident on the second experimental day.

Overall, the ingestion of dairy milk with a phosphorus chelator resulted in a faster, sharper, and more prolonged postprandial glucose rise compared with milk consumed with placebo, despite comparable baseline and peak glucose concentrations. **Figures 3.17** and **3.18** illustrate the mean blood glucose concentrations and the mean ΔBG changes, respectively, across the treatment groups on both intervention Days 1 and 2.

Table 3.14. Postprandial glucose dynamics following test meal: concentrations, peak time, magnitude, and duration over 180 minutes on Days 1 and 2.

	Placebo	Chelator	*p*-value
Baseline glucose (mg/dl)			
Day 1	110.0 ± 2.3	106.1 ± 3.6	0.229
Day 2	108.7 ± 2.8	104.0 ± 3.2	0.093
Peak glucose (mg/dl)			
Day 1	120.0 ± 2.5	123.1 ± 4.5	0.417
Day 2	116.3 ± 4.2	120.4 ± 4.3	0.145
Time to peak (min)			
Day 1	106.9 ± 12.2	67.5 ± 8.9	**0.021***
Day 2	98.0 ± 13.2	72.0 ± 11.6	**0.041***
Peak glucose increment (mg/dl)			
Day 1	10.0 ± 1.6	17.0 ± 2.2	**0.006***
Day 2	7.5 ± 2.2	16.4 ± 1.9	**<0.001***
ΔBG at 30 min (mg/dl)			
Day 1	1.0 ± 1.2	12.1 ± 2.0	**<0.001***
Day 2	1.7 ± 2.4	9.5 ± 2.6	**0.002***
ΔBG at 60 min (mg/dl)			
Day 1	1.9 ± 2.6	8.9 ± 3.0	**0.038***
Day 2	-2.2 ± 2.5	6.6 ± 2.1	**0.015***
ΔBG at 90 min (mg/dl)			
Day 1	0.4 ± 2.4	7.2 ± 2.4	**0.012***
Day 2	-0.3 ± 2.9	7.5 ± 1.8	**0.028***
ΔBG at 120 min (mg/dl)			
Day 1	0.6 ± 1.4	4.1 ± 2.5	**0.044***
Day 2	-2.0 ± 2.4	5.5 ± 2.1	**0.041***
ΔBG at 150 min (mg/dl)			
Day 1	-0.3 ± 1.7	1.1 ± 2.5	0.5852
Day 2	-6.8 ± 2.0	6.5 ± 2.0	**0.001***
ΔBG at 180 min (mg/dl)			
Day 1	-4.8 ± 2.2	-1.8 ± 2.8	0.359
Day 2	-6.1 ± 2.0	1.0 ± 2.1	**0.017***
Positive iAUC (mg·min/dl)			
Day 1	522.2 ± 107.8	1,300.3 ± 210.6	**0.004***
Day 2	463.1 ± 134.8	1,260.0 ± 143.1	**<0.001***
Negative iAUC (mg·min/dl)			
Day 1	-487.5 ± 68.6	-326.3 ± 85.1	0.155
Day 2	-795.0 ± 142.0	-175.0 ± 74.5	**0.004***

ΔBG, changes in blood glucose levels; iAUC, incremental area under the curve; min, minutes.
Data presented as mean ± standard error of the mean (SEM). A 2-tailed paired t-test was performed between groups. *Indicates statistical significance ($p < 0.05$).

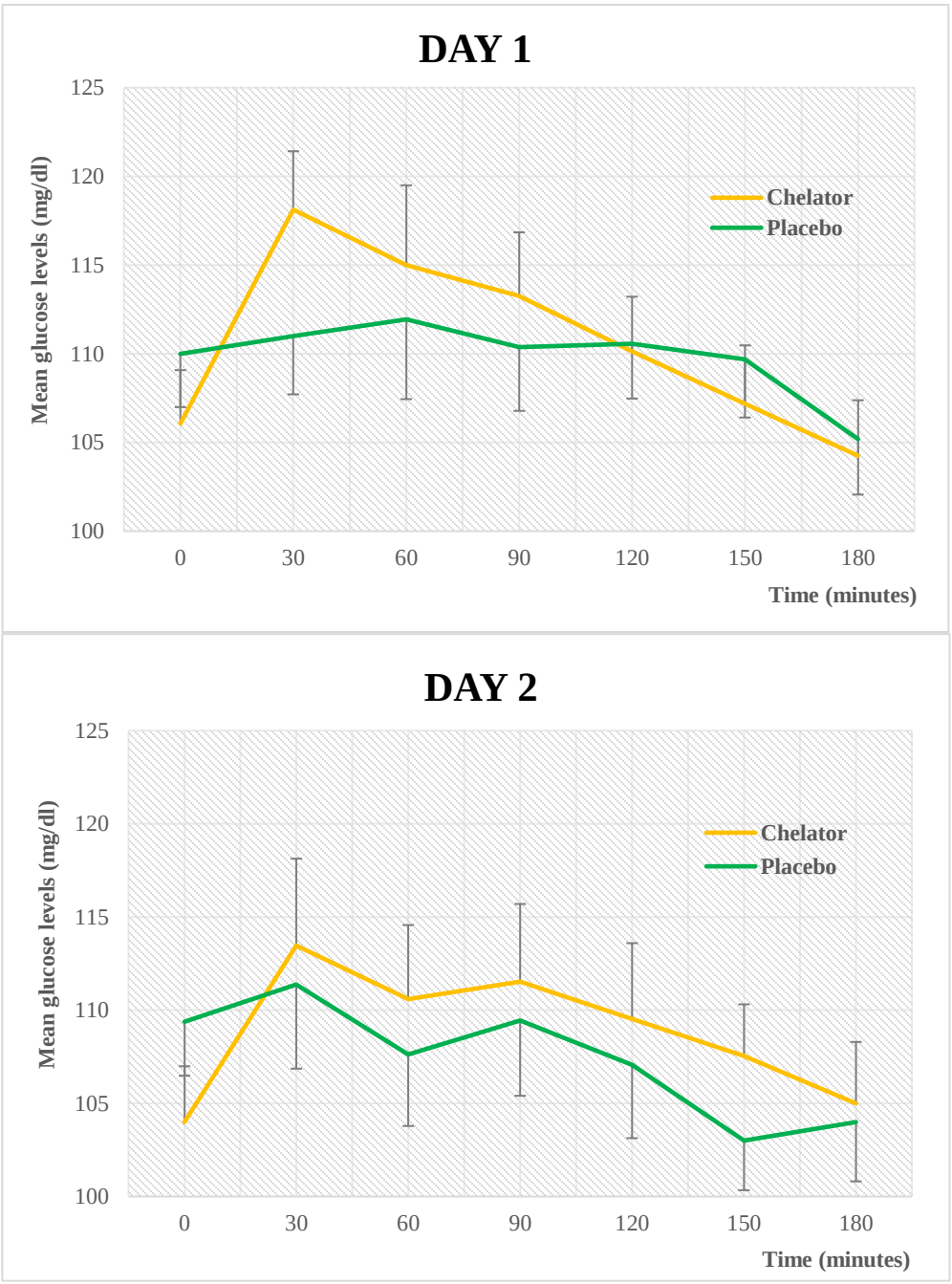


Figure 3.17. Time course of postprandial blood glucose response following the test meal on intervention Days 1 and 2.
Vertical bars indicate standard error of the mean (SEM).

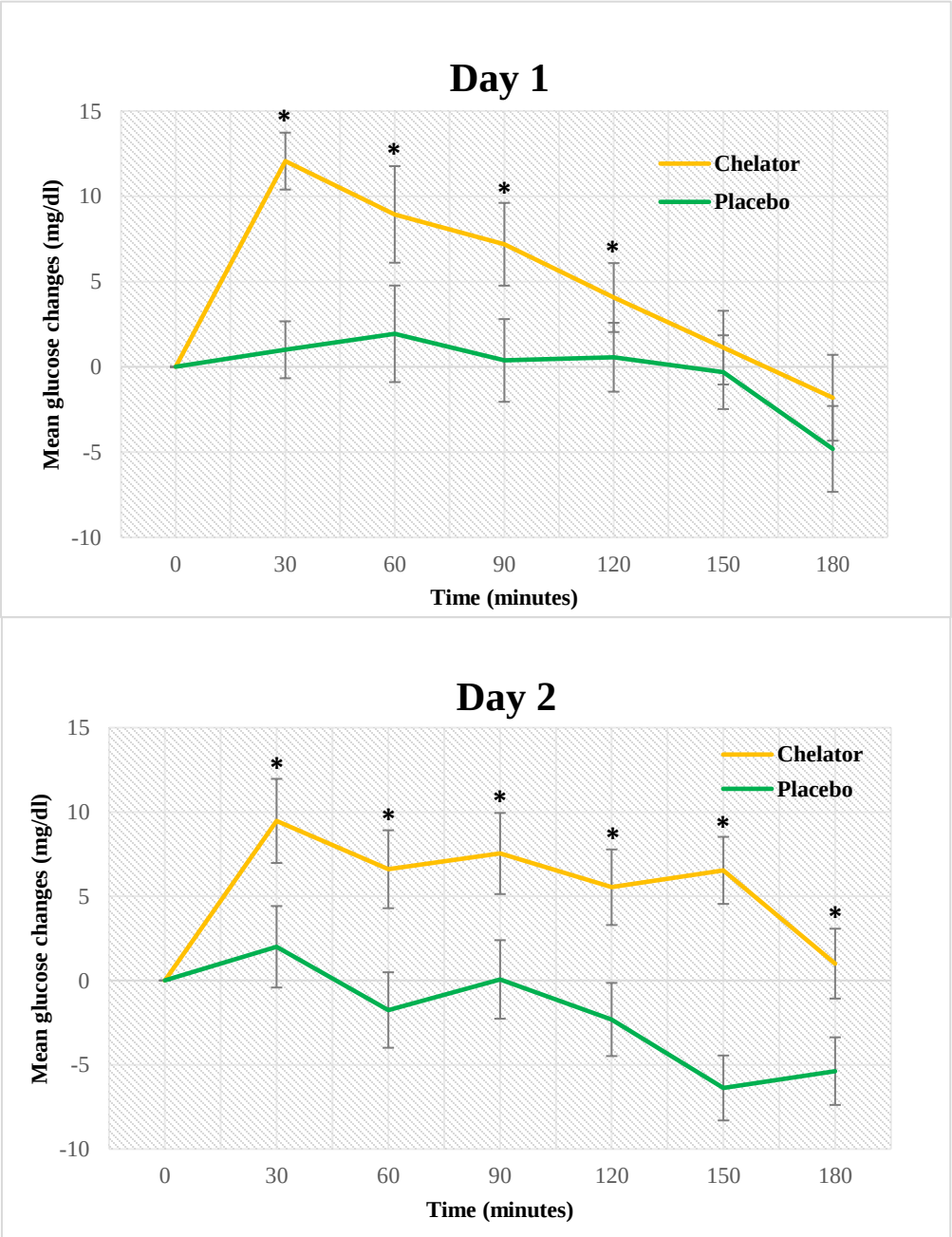


Figure 3.18. Time course of postprandial changes in blood glucose (ΔBG) in response to the test meal across intervention Days 1 and 2.

Vertical bars indicate standard error of the mean (SEM).

*Indicates a statistically significant difference between both groups ($p < 0.05$).

3.5.3 Postprandial core body temperature

A multivariate repeated measures ANOVA was conducted to examine the effects of time, day, treatment, and their interactions on postprandial CBT. The analysis indicated that CBT remained largely stable over time, as the main effect of time was not significant ($p = 0.164$). Similarly, no significant differences were observed between treatment groups, with estimated marginal means of 0.067 ± 0.033 °C for the placebo group and 0.064 ± 0.034 °C for the chelator group (mean difference = -0.003 °C, $p = 0.955$). The interaction between time and treatment was also non-significant ($p = 0.249$), indicating that dairy-derived phosphorus intake did not influence the temporal pattern of CBT. Additionally, the day factor did not significantly affect CBT changes over time (time × day interaction, $p = 0.247$), and the three-way interaction among time, treatment, and day was likewise non-significant ($p = 0.667$).

In a follow-up univariate analysis, CBT variations were assessed for both phosphorus and chelator groups across Days 1 and 2 using a bivariable analysis. Baseline CBT values were comparable between the two interventions on both days (Day 1: 37.00 ± 0.05 vs. 37.00 ± 0.06 °C, mean difference = 0.001, $p = 0.978$; Day 2: 37.01 ± 0.11 vs. 37.04 ± 0.08 °C, mean difference = -0.037, $p = 0.754$), indicating similar starting points for all participants prior to meal consumption.

Following the test meal, postprandial CBT changes at all measured time points (30, 60, 90, 120, 150, and 180 minutes) did not differ significantly between phosphorus and chelator groups on either day, as illustrated in **Table 3.15**. On Day 1, CBT deltas ranged from 0.02 to 0.06 °C for the phosphorus group and 0.06 to 0.11 °C for the chelator group, with mean

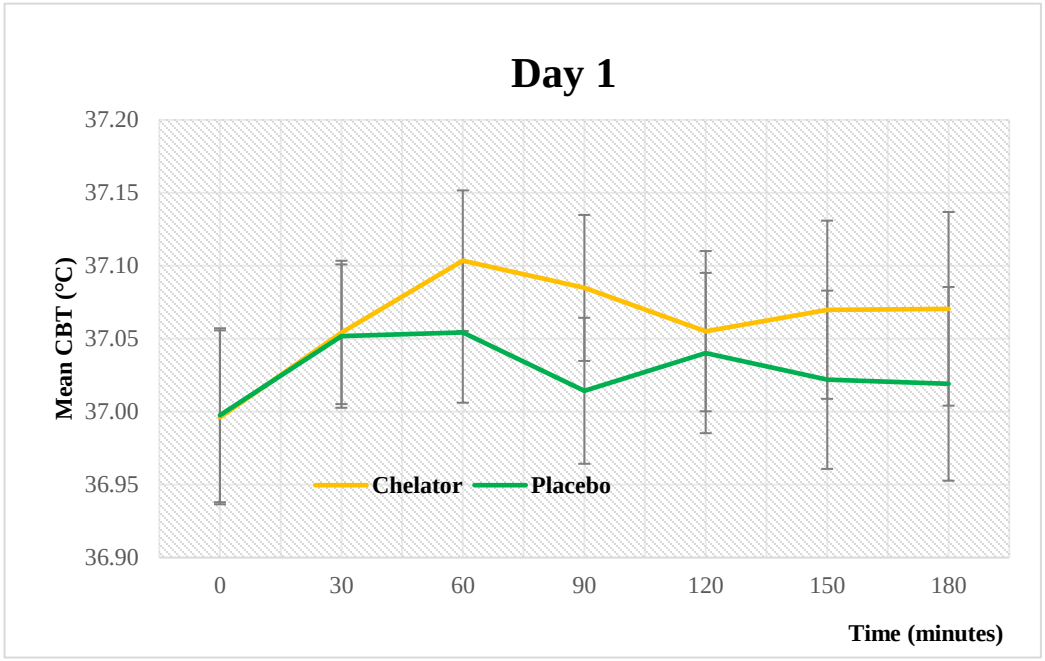
differences at each time point ranging from -0.004 to -0.072 °C (all $p > 0.28$). Similarly, on Day 2, CBT deltas ranged from 0.09 to 0.16 °C in the phosphorus group and 0.01 to 0.11 °C in the chelator group, with mean differences between groups from -0.005 to 0.086 °C (all $p > 0.27$).

The greatest absolute postprandial CBT change was observed at 150 minutes on Day 2 in the phosphorus group (0.16 ± 0.10 °C), although this increase did not reach statistical significance compared with the chelator group (0.08 ± 0.08 °C, mean difference = 0.086, $p = 0.515$). Overall, the magnitude and temporal pattern of CBT responses were highly similar between groups on both days, and there was no consistent trend favoring either intervention at any specific time point. **Figures 3.19** and **3.20** provide a visual representation of the mean CBT and mean ΔCBT over time for both treatment groups.

Table 3.15. Mean postprandial core body temperature variations over 180 minutes on Days 1 and 2.

	Chelator	Placebo	*p*-value
CBT at baseline (°C)			
Day 1	37.00 ± 0.06	37.00 ± 0.05	0.978
Day 2	37.04 ± 0.08	37.01 ± 0.11	0.754
ΔCBT at 30 min (°C)			
Day 1	0.06 ± 0.05	0.05 ± 0.04	0.948
Day 2	0.01 ± 0.04	0.08 ± 0.06	0.273
ΔCBT at 60 min (°C)			
Day 1	0.11 ± 0.05	0.06 ± 0.05	0.364
Day 2	0.07 ± 0.06	0.09 ± 0.08	0.799
ΔCBT at 90 min (°C)			
Day 1	0.09 ± 0.06	0.02 ± 0.05	0.285
Day 2	0.10 ± 0.05	0.10 ± 0.08	0.953
ΔCBT at 120 min (°C)			
Day 1	0.06 ± 0.06	0.04 ± 0.04	0.795
Day 2	0.11 ± 0.06	0.11 ± 0.08	0.939
ΔCBT at 150 min (°C)			
Day 1	0.07 ± 0.06	0.02 ± 0.05	0.490
Day 2	0.08 ± 0.08	0.16 ± 0.1	0.515
ΔCBT at 180 min (°C)			
Day 1	0.07 ± 0.05	0.02 ± 0.06	0.471
Day 2	0.07 ± 0.08	0.09 ± 0.09	0.852

ΔCBT, changes in core body temperature; °C, degree Celsius.
Data presented as mean ± standard error of the mean (SEM). A 2-tailed paired t-test was performed between groups.



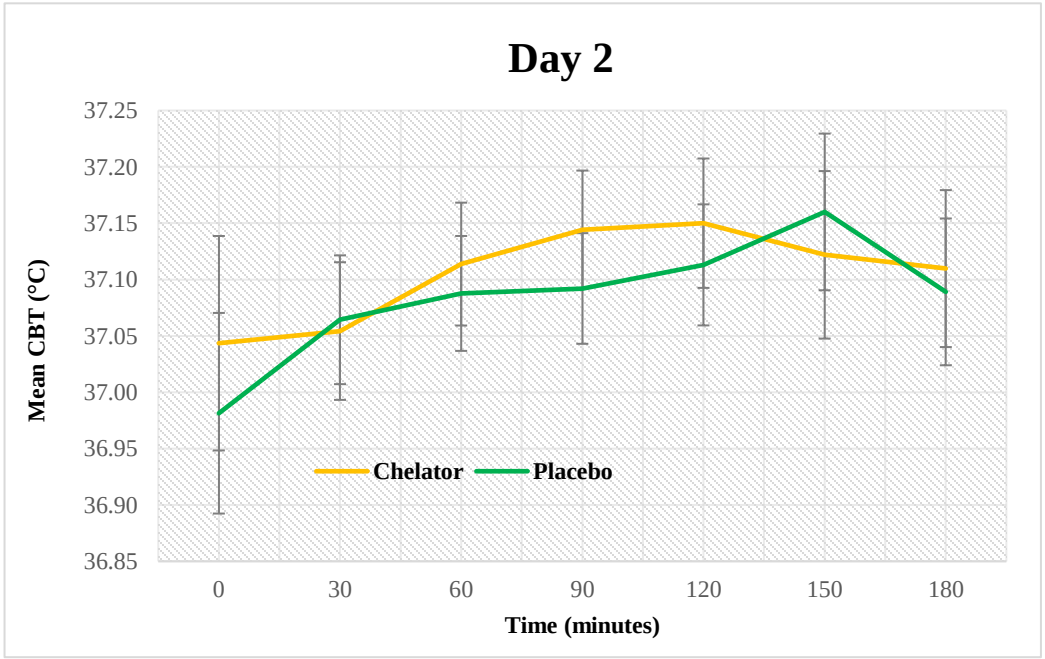


Figure 3.19. Time course of postprandial core body temperature in response to the test meal across intervention Days 1 and 2.
Vertical bars indicate standard error of the mean (SEM).

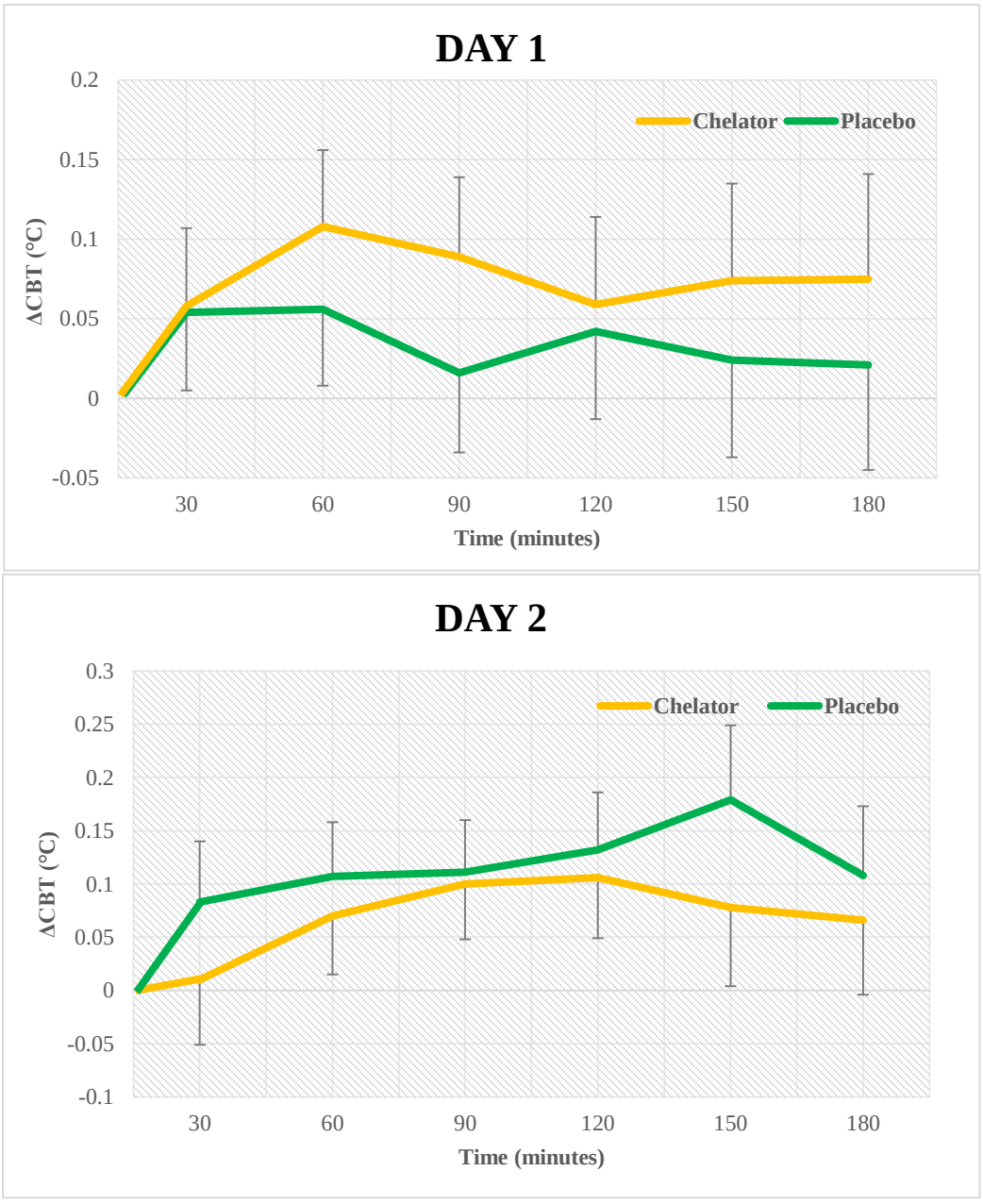


Figure 3.20. Time course of postprandial changes in core body temperature (ΔCBT) in response to the test meal across intervention Days 1 and 2.
Vertical bars indicate standard error of the mean (SEM).

3.5.4 Appetite and food preference assessments

Subjective appetite and food preference assessments were conducted using the visual analog scale (VAS). Assessments were recorded at baseline and subsequently at 30-minute intervals over a three-hour period following meal ingestion. All 16 participants completed the questionnaire on both days of each intervention.

A three-way repeated measures ANOVA was conducted to evaluate the effects of time, treatment, and their interaction on subjective appetite sensations (hunger, satiety, fullness, and prospective food consumption (PFC)) as well as on specific food preferences (sweet, salty, fatty, and savory) across the two intervention days (**Table 3.16**).

The analysis revealed a highly significant main effect of time for all appetite-related outcomes, including hunger, satiety, fullness, and prospective food consumption (PFC), across both intervention days (all $p \leq 0.009$). This consistent time effect indicates that subjective sensations of appetite varied significantly during the postprandial period following meal ingestion, reflecting the expected physiological response to feeding. Specifically, appetite scores fluctuated markedly within the three-hour postprandial window, suggesting dynamic shifts in hunger suppression and satiety development over time irrespective of treatment condition.

Similarly, all food preference variables, sweet, salty, fatty, and savory, also demonstrated significant main effects of time (all $p \leq 0.033$), indicating that taste-related perceptions and preferences were not static but changed significantly throughout the postprandial period. This temporal variation suggests that the hedonic evaluation of different food attributes evolved in

response to meal consumption and subsequent satiety signals, a pattern consistent across both test days.

In contrast, the main effect of treatment was generally not significant for most outcomes, suggesting that treatment alone did not produce uniform shifts in subjective ratings when averaged across all time points. The only exception was observed for satiety on Day 1 ($p = 0.009$), where a significant treatment effect was detected, indicating that phosphorus supplementation transiently influenced satiety perception on the first intervention day. All other appetite and taste preference measures showed non-significant treatment effects (all $p > 0.1$), highlighting that, in isolation, the intervention did not consistently alter mean appetite or sensory preference levels.

However, the time × treatment interaction yielded a distinctly different pattern. Highly significant interactions were observed for hunger, satiety, fullness, and PFC on both intervention days (all $p \leq 0.003$), indicating that the trajectory of postprandial appetite responses over time varied markedly between treatment conditions. Notably, the differences that reached statistical significance primarily occurred during the later postprandial period (**Figures 3.20** and **3.21**). Hunger differences were most pronounced at 150 and 180 minutes, satiety at 120, 150, and 180 minutes, while fullness and PFC differences were mainly observed between 150 and 180 minutes.

For the food preference measures, the pattern was comparatively stable across treatments. No significant time × treatment interactions were detected for sweet, salty, savory or fatty taste preferences on either day (all $p > 0.1$), indicating that postprandial changes in these hedonic responses followed similar temporal trends in both groups.

Overall, these results indicate that time was the predominant factor influencing postprandial changes in both appetite sensations and food preference ratings. While treatment alone had limited effects, the significant time × treatment interactions for key appetite measures highlight the presence of distinct temporal dynamics in appetite regulation between the two interventions, with the phosphorus condition producing a different postprandial trajectory of hunger, satiety, fullness, and food desire compared with placebo.

Table 3.16. Repeated measures ANOVA for postprandial subjective appetite ratings and food preferences over 120 minutes across two intervention days.

Outcome variables	Time (p value)	Treatment (p value)	Time x treatment (p value)
Hunger			
Day 1	**0.002***	0.371	**< 0.0001***
Day 2	**0.009***	0.813	**< 0.0001***
Satiety			
Day 1	**< 0.0001***	**0.009***	**< 0.0001***
Day 2	**< 0.0001***	0.691	**< 0.0001***
Fullness			
Day 1	**< 0.0001***	0.147	**<0.0001***
Day 2	**< 0.0001***	0.419	**<0.0001***
PFC			
Day 1	**< 0.0001***	0.153	**0.003***
Day 2	**< 0.0001***	0.733	**0.045***
Sweet			
Day 1	**0.002***	0.559	0.161
Day 2	**0.006***	0.399	0.715
Salty			
Day 1	**0.033***	0.628	0.738
Day 2	**0.001***	0.114	0.352
Fatty			
Day 1	**<0.001***	0.539	0.127
Day 2	**0.005***	0.698	0.188
Savory			
Day 1	**0.004***	0.654	0.126
Day 2	**0.012***	0.568	0.340

PFC, prospective food consumption.

* Indicates statistical significance ($p < 0.05$) based on repeated measures ANOVA.

Figure 3.21. Changes in subjective appetite ratings (hunger and satiety) from baseline to 180 minutes following test meal ingestion across intervention Days 1 and 2.

Vertical bars indicate standard deviation.

*Indicates a statistically significant difference between both groups ($p < 0.05$).

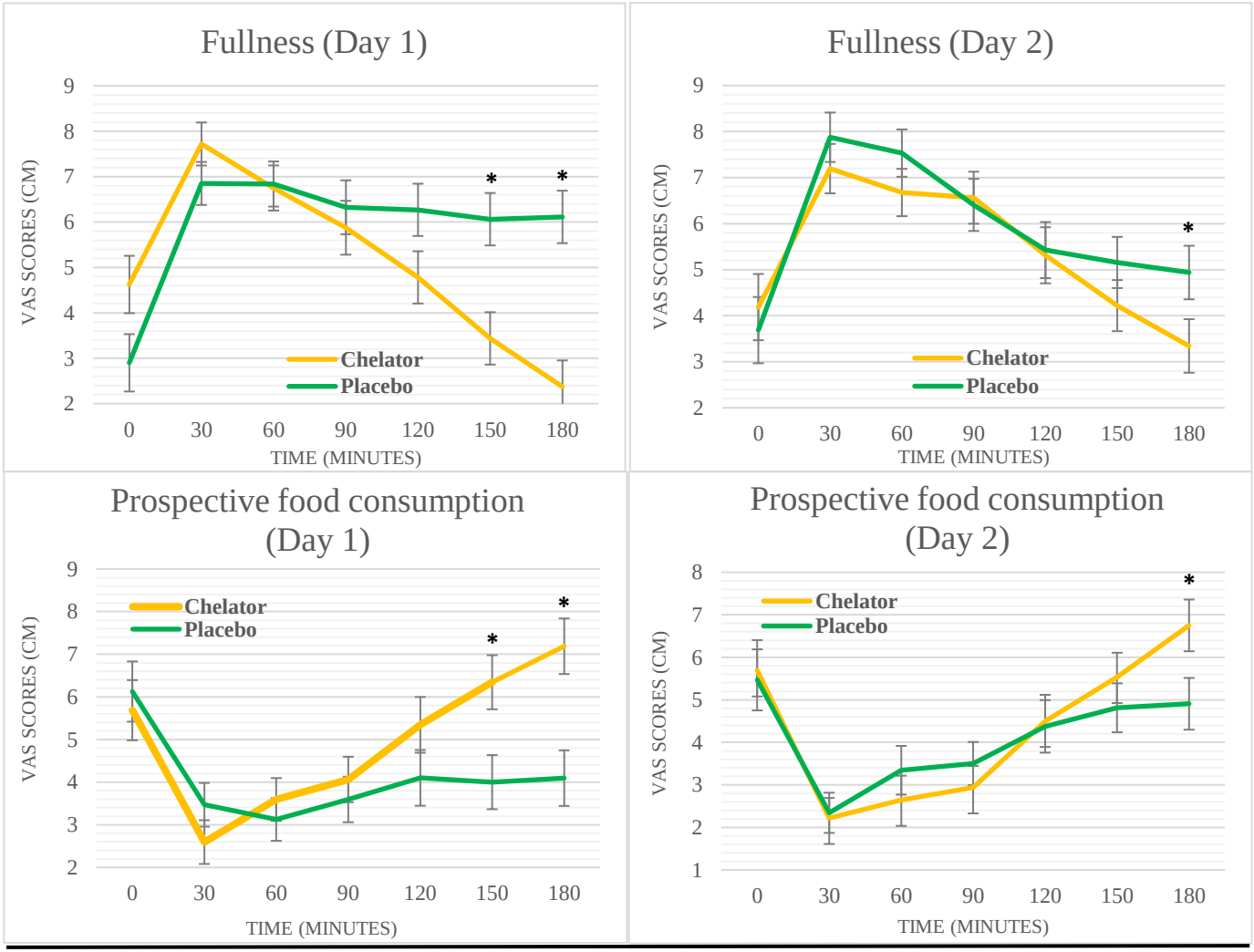


Figure 3.22. Changes in subjective appetite ratings (fullness and prospective food consumption) from baseline to 180 minutes following test meal ingestion across intervention Days 1 and 2.
Vertical bars indicate standard deviation.
*Indicates a statistically significant difference between both groups ($p < 0.05$).

3.6 Discussion (Experiments 1 and 2)

This dual study investigated the effects of dietary phosphorus on postprandial glucose response, core body temperature (CBT), and appetite-related measures in healthy individuals. Designed to complement each other, Experiment 1 assessed the impact of phosphorus supplementation, administered as 685 mg of phosphorus in the form of potassium phosphate, while Experiment 2 examined the effects of naturally occurring dairy-derived phosphorus. Together, these experiments provide an integrated analysis of how different forms of dietary phosphorus influence glucose regulation, thermogenesis, and appetite in healthy individuals. Overall, the findings from both experiments demonstrated that dietary phosphorus significantly influenced postprandial glucose metabolism and appetite regulation, but had no impact on CBT, suggesting that dietary phosphorus may not have directly influenced thermogenic responses under the conditions of this study. The subsequent sections will provide a detailed interpretation of these effects, while offering a comprehensive analysis of the findings.

3.6.1. Dietary phosphorus and postprandial glucose regulation

In Experiment 1, phosphorus supplementation markedly influenced postprandial glucose metabolism. This was evidenced by significantly lower peak glucose increments, reduced iAUC, and faster return to baseline glucose levels in the phosphorus group compared to placebo. Blood glucose levels remained lower throughout the postprandial period in the phosphorus condition, with statistically significant differences at 30 and 60 minutes, reflecting an early modulatory effect. The transient nature of this effect suggests that phosphorus primarily impacts the early stages of glucose

clearance, potentially through enhanced insulin-mediated glucose disposal or increased hepatic glucose uptake.

Beyond 90 minutes, differences were not statistically significant, although glucose values remained numerically lower, suggesting that endogenous glucose homeostasis mechanisms, including insulin secretion and hepatic glucose regulation, equilibrated between groups over time. Peak glucose increments further illustrate that phosphorus mitigates postprandial glucose spikes, while lower positive iAUC values indicate attenuated glucose excursions, reflecting improved glycemic control. Faster return to baseline in the phosphorus group, within 60 minutes versus 90 minutes in placebo, provides additional support for the role of phosphorus in facilitating efficient glucose clearance. Collectively, these findings underscore the involvement of phosphorus in glucose metabolism, insulin signaling, and ATP production, highlighting its potential therapeutic value for enhancing postprandial glucose regulation.

As phosphorus was administered in the form of potassium phosphate, the potential contribution of potassium, known to enhance insulin secretion and sensitivity, must also be considered. To isolate the specific effects of phosphorus, Experiment 2 employed a phosphorus chelator that selectively binds phosphorus, allowing assessment of its role independent of potassium or other interactions. This methodological approach confirmed and extended the results from Experiment 1, demonstrating that phosphorus exerts a significant, favorable impact on postprandial glucose metabolism.

In Experiment 2, the chelator condition produced significantly larger and more rapid postprandial glucose excursions compared with placebo milk. Time to peak glucose was accelerated by 37–45%, while peak glucose increments from baseline increased by 70–120%, indicating both a faster and sharper rise in blood glucose. Furthermore, the iAUC was substantially higher in the chelator group, and glucose clearance was delayed, requiring up to 180 minutes to return to baseline compared to a much faster recovery in the placebo group. These findings highlight the physiological consequences of reduced phosphorus availability, including impaired early-phase glucose regulation and prolonged hyperglycemia. They also underscore the temporal importance of phosphorus: availability influences not only the magnitude of postprandial glucose fluctuations but also the timing of glucose peaks and recovery, with potential implications for dietary management and clinical interventions targeting postprandial glycemia.

The accelerated glucose peaks, delayed return to baseline, and increased iAUC in the chelator group support the hypothesis that phosphorus is integral to cellular glucose uptake via insulin signaling and subsequent intracellular phosphorylation. When phosphorus is unavailable, the formation of glucose-6-phosphatec, a key intermediate in glycolysis and glycogen synthesis, is hindered, delaying glucose metabolism, producing higher peaks, and slowing clearance. These results emphasize phosphorus's pivotal role in maintaining glucose homeostasis and highlight its contribution to early-phase postprandial glycemic control.

Together, the findings from Experiments 1 and 2 demonstrate that dietary phosphorus significantly modulates postprandial glucose dynamics, with effects contingent on its form and availability. In Experiment 1,

supplementation attenuated peak glucose levels, glucose excursions, iAUC values, and postprandial responses at 30 and 60 minutes. In Experiment 2, phosphorus chelation amplified and prolonged glucose excursions, delayed glucose clearance, increased iAUC, and elevated postprandial glucose responses across the entire two-hour postprandial period. Collectively, these results emphasize that dietary phosphorus, whether supplemented or restricted, plays a central role in regulating postprandial glycemia.

These experimental outcomes are consistent with previous animal and human studies. In rats, phosphorus restriction induces insulin resistance and hyperglycemia, whereas phosphorus-rich diets improve glucose regulation (Abuduli et al., 2016; Xie et al., 2000). Observational human studies indicate that serum phosphate levels are negatively correlated with postprandial glucose concentrations and HOMA-IR, positively associated with insulin sensitivity, but not significantly linked to insulin secretion (Haglin et al., 2001; Haap et al., 2006; Khattab et al., 2015; Akter et al., 2020). Phosphate infusion in healthy subjects improves insulin sensitivity under euglycemic conditions (Nowicki et al., 1996). Similarly, Khattab et al. demonstrated that co-ingestion of phosphorus (500 mg) with a 75 g glucose solution improved postprandial glucose by 5 mg/dl at 60 minutes. In the current study, Experiment 1 showed a 16 mg/dl improvement and Experiment 2 an 8 mg/dl improvement at the same time point, further supporting the role of extracellular phosphorus in facilitating phosphorylation-dependent glucose metabolism.

Experiment 2 also aligns with evidence on the glycemic buffering effects of milk and dairy products. Naturally rich in phosphorus, dairy elicits low postprandial glucose responses despite low glycemic indices, in part through insulinotropic whey proteins and branched-chain amino acids,

which stimulate postprandial insulin secretion and release of gut hormones such as GLP-1, PYY, and CCK, regulating glucose and satiety (Ostman et al., 2001; Elmståhl & Björck, 2001; Nilsson et al., 2004; Zawadzki et al., 1992; Loon et al., 2000; Luhovyy et al., 2007). However, recent evidence indicates that dairy's glycemic effects may reflect improved insulin sensitivity rather than increased insulin secretion (Kung et al., 2020; El Khoury et al., 2014). Collectively, these studies support the hypothesis that phosphorus, rather than lactose or protein content alone, is a major contributor to milk's postprandial glycemic regulation. This is confirmed by the chelator intervention, where phosphorus binding significantly elevated glucose levels, demonstrating a causal relationship between dairy-derived phosphorus and postprandial glycemia. The findings underscore phosphorus's critical role in stabilizing glucose responses and highlight its potential contribution to the insulinotropic and glycemic-buffering properties of dairy products.

3.6.2 Dietary phosphorus and diet induced thermogenesis: Implications beyond core body temperature

Despite the significant effect of dietary phosphorus on postprandial glucose metabolism, no significant effect of dietary phosphorus on CBT was observed in either of the experiments. Since CBT reflects metabolic heat production, it is possible that the thermogenic effects of phosphorus supplementation, as assessed in Experiment 1, and phosphorus chelation, as examined in Experiment 2, were either too subtle to be detected or compensated for by other physiological mechanisms. Furthermore, even though the test meal in Experiment 1 was high in protein, a known contributor to DIT, it did not appear to influence CBT in this context.

These findings contrast with previous research, which has consistently reported a positive association between phosphorus intake and DIT (Bassil et al, 2016; Abdouni et al, 2018; Assaad et al, 2018). Additionally, several studies have shown that food intake increases CBT in response to metabolic processes such as digestion, absorption, and nutrient metabolism, regardless of phosphorus availability (Heikens et al, 2011; Hoffmann et al, 2012; McGann et al, 1993; Reinhardt et al, 2016; Rising et al, 1992; Vinales et al, 2019; Westerterp-Plantenga et al, 1990). However, despite these consistent findings, the results from this dual study did not align with the established literature. Several potential explanations may account for the observed discrepancies.

First, the CORE device used in both experiments, while practical and non-invasive, may have lower sensitivity and accuracy compared to gold-standard methods like rectal or esophageal thermometry, which could have impacted the ability to detect subtle changes in CBT (Hymczak et al, 2021). In this regard, some studies have noted that wearable body surface temperature measurements are often more indicative of skin temperature rather than core body temperature (Petrone et al, 2014; Koop & Tadi, 2023). Second, it is important to consider the limitations of using CBT as a proxy for DIT. In previous studies, gold-standard methods such as indirect calorimetry were typically used to assess the relationship between phosphorus and DIT (Bassil et al, 2016; Abdouni et al, 2018; Assaad et al, 2018). Indirect calorimetry measures oxygen consumption and carbon dioxide production to estimate energy expenditure, providing a precise evaluation of metabolic shifts following food intake. This study, however, utilized CBT as a proxy for thermogenesis. Although CBT can reflect changes in thermogenic activity, it may not be sufficiently sensitive to

detect the subtle metabolic shifts induced by dietary interventions, particularly when compared to the sensitivity of indirect calorimetry. Moreover, thermogenesis is a complex process involving multiple pathways, and its effects on body temperature may be diluted by factors such as individual variability in thermoregulatory responses, meal composition, and ambient environmental conditions.

Third, one of the most important explanations for the discrepancy observed in both experiments is the variability in meal ingestion time. While participants were instructed to consume the test meal within 10-15 minutes, some took considerably longer, which may have delayed the thermogenic response and shifted it beyond the primary measurement window. DIT typically exhibits a delayed response, as the increase in energy expenditure does not occur immediately upon food consumption but develops progressively over time, due to the physiological processes of digestion, nutrient absorption, and metabolic utilization. This delayed response has been observed in both human and animal studies, highlighting the transient nature of DIT following food intake (Nedergaard et al, 2023; Speakman & Kejjer, 2021). Given that DIT is characterized by higher peaks occurring after meal consumption, prolonged ingestion time may have shifted and weakened the thermogenic response, potentially obscuring its full magnitude within the designated observation period.

Fourth, the acute nature of this study may have contributed to the lack of observed effect. Previous studies demonstrating an association between phosphorus and DIT often involved chronic supplementation or long-term dietary modifications, which may elicit more pronounced thermogenic effects (Abdouni et al, 2018). In contrast, the present study assessed acute postprandial responses over a single meal, potentially limiting the ability to

detect cumulative or adaptive thermogenic changes. Despite the lack of significant effects on CBT, phosphorus supplementation and chelation did have a notable impact on appetite measures in both experiments, reducing hunger and prospective food consumption (PFC) while enhancing satiety and fullness. This suggests that phosphorus may have influenced energy metabolism through mechanisms not directly reflected in CBT. Specifically, phosphorus may have enhanced DIT at a cellular level by increasing ATP availability and energy expenditure postprandially, without significantly raising whole-body CBT. Phosphorus is a key component of ATP, and an increase in ATP production could drive metabolic processes contributing to satiety without manifesting as a rise in CBT. Additionally, hepatic ATP levels are involved in signaling pathways related to energy balance and appetite regulation, suggesting that phosphorus may have exerted its effects by modulating hepatic energy metabolism rather than through systemic thermogenic responses detectable via CBT.

The observed appetite changes in both experiments align with previous research demonstrating that dietary phosphorus can enhance postprandial energy metabolism and reduce food intake, potentially due to its role in mitochondrial ATP synthesis and hypothalamic signaling (Obeid et al, 2010; Ayoub et al, 2015). For instance, Bassil et al. found that phosphorus supplementation induced a significant 23% increase in DIT area under the curve (measured via substrate oxidation), accompanied by a reduction in appetite as measured through VAS questionnaires (Bassil et al, 2016). Although direct thermogenic effects might not have been captured through CBT, the appetite-related changes in both experiments suggest that phosphorus may have influenced energy balance through mechanisms other than whole-body thermogenesis, potentially involving hepatic ATP

dynamics and mitochondrial efficiency. Further research using more sensitive methods, such as indirect calorimetry, and a longer supplementation period is warranted to better evaluate the role of phosphorus in DIT and energy metabolism.

3.6.3 Dietary phosphorus and appetite regulation

Appetite modulation following phosphorus interventions showed both similarities and temporal differences between experiments. In Experiment 1, phosphorus supplementation significantly influenced postprandial appetite dynamics, producing lower hunger and PFC scores, while enhancing satiety and fullness relative to placebo. These effects emerged early, from 30–45 minutes post-meal, and persisted throughout the postprandial period, demonstrating a robust appetite-suppressive effect of phosphorus. However, specific food preferences (sweet, salty, fatty, savory) were largely unaffected by treatment, with temporal declines in desire for these foods occurring independently of phosphorus intake.

Given that the test meal in Experiment 1 contained 32% protein, an established appetite-regulating macronutrient, the observed effects were unlikely to be driven solely by protein content but also by phosphorus. This is particularly evident considering that the egg white powder used as the protein source contained negligible amounts of phosphorus. Despite both groups consuming the same high-protein meal, significant differences in appetite measures were still observed between the supplemented and placebo groups. This indicates that the appetite-modulating effects were primarily attributable to phosphorus, reinforcing its role in appetite regulation beyond that of dietary protein. As with glucose metabolism, the potential influence of potassium cannot be dismissed. Although the

mechanisms by which potassium affects appetite remain unclear, some evidence suggests it may contribute to weight regulation by modulating appetite and cellular energy balance (Tal et al., 2019). However, the use of a phosphorus chelator in Experiment 2 allowed for the isolation of phosphorus-specific effects. The significant appetite-related outcomes observed in Experiment 2 indicate that the effects seen in Experiment 1 were attributable to phosphorus rather than potassium.

In Experiment 2, the overall pattern of appetite responses was broadly similar to Experiment 1, though temporal dynamics differed. Significant time × treatment interactions were observed for hunger, satiety, fullness, and PFC, with differences most pronounced in the later postprandial period (120–180 minutes). In contrast to Experiment 1, the main effect of treatment was generally minimal, suggesting that phosphorus chelation did not uniformly alter average appetite ratings but did influence the trajectory of postprandial appetite responses. As in Experiment 1, food preference ratings remained largely unaffected by treatment.

These findings suggest that phosphorus plays a crucial role in modulating appetite-related pathways, potentially via its involvement in ATP production. Since phosphorus is essential for ATP synthesis, its chelation may have reduced ATP availability, thereby impairing energy metabolism and triggering compensatory mechanisms that enhance hunger signaling. The results align with previous research, which has shown that dietary phosphorus influences metabolic rates and energy expenditure, further supporting its role in appetite regulation. Notably, studies by Obeid et al. demonstrated that adding phosphorus to carbohydrate preloads reduced subsequent ad libitum energy intake in lean individuals (Obeid et al, 2010), while Ayoub et al. reported that consuming 375 mg of phosphorus per meal

over three months led to reduced appetite and food intake in individuals with obesity (Ayoub et al, 2015). These findings collectively suggest that phosphorus availability, whether through supplementation or chelation, directly influences appetite regulation via energy metabolism mechanisms. Despite the significant impact of phosphorus on appetite regulation, neither supplementation in Experiment 1 nor chelation in Experiment 2 appeared to influence subjective food preferences for sweet, salty, fatty, or savory foods. In both studies, variations in food preferences were primarily driven by time rather than phosphorus availability, with postprandial fluctuations in cravings occurring naturally. This aligns with previous research indicating that food preferences are more strongly influenced by palatability, sensory perception, and hedonic responses rather than micronutrient-induced metabolic changes (Forde & de Graaf, 2022; Berthoud, 2011). Consequently, these findings suggest that the role of phosphorus in appetite control is metabolic rather than sensory in nature.

3.6.4 Strengths and limitations

A key strength shared by both experiments is the crossover design, which allowed each participant to serve as their own control. This design minimized inter-individual variability and provided a clearer comparison of the effects of phosphorus exposure within the same individuals. Additionally, the consistency in results across both test days (Day 1 and Day 2) in both studies reinforces the reliability of the findings, confirming that the experimental outcomes were reproducible and not influenced by test timing, test days, or experimental design.

Another notable strength of both studies was the use of continuous monitoring devices for glucose (DEXCOM) and core body temperature

(CORE). These devices provided real-time, high-resolution data collection, capturing the dynamic nature of postprandial metabolic responses. Unlike studies that rely on intermittent blood sampling, this approach enhanced the precision and accuracy of glucose fluctuations and thermogenic responses, contributing to a more comprehensive understanding of postprandial metabolism.

A specific strength of Experiment 2 was the use of chelation, which enabled the establishment of a controlled phosphorus-restricted condition. To the best of our knowledge, this is the first study to investigate postprandial responses under conditions of phosphorus restriction. This approach allowed for the precise isolation of the independent effect of dietary phosphorus on glucose metabolism and thermogenesis, effectively minimizing the potential confounding influence of other minerals such as potassium or calcium. Additionally, testing naturally occurring dairy-derived phosphorus enhances the real-world applicability of the findings, as it more closely reflects the effects of phosphorus from dietary sources rather than synthetic phosphorus.

Despite the methodological strengths, several limitations should be considered. One potential limitation of both studies is the short duration of phosphorus supplementation. Given the acute nature of the intervention, longer-term studies may be necessary to determine whether phosphorus has sustained effects on thermogenesis and appetite regulation.

Another common limitation is the relatively small sample size (N = 16). Although the crossover design helped mitigate individual differences, a larger sample size would have increased statistical power to detect subtle changes, particularly in thermogenic responses.

Additionally, while the use of continuous monitoring devices improved data accuracy, core body temperature (CBT), as mentioned earlier, may not serve as a sensitive proxy for diet-induced thermogenesis (DIT) compared with gold-standard methods such as indirect calorimetry. As a result, subtle thermogenic effects of phosphorus may have been overlooked.

A further limitation of both studies is the variability in ingestion time among participants consuming the dairy test drink. Differences in the duration taken to consume the test meal could have influenced postprandial responses, introducing an additional source of variability. Future studies should standardize ingestion time to ensure greater consistency across participants.

Moreover, the pH of the test meals was not standardized or recorded. Since phosphorus absorption can be influenced by luminal acidity and the solubility of phosphate salts, variations in meal pH could have affected the rate and extent of phosphorus absorption, potentially contributing to inter-individual variability in postprandial metabolic outcomes.

The absence of insulin measurements also represents a critical limitation. Insulin plays a central role in carbohydrate metabolism, and its assessment would have provided insight into both insulin production and sensitivity. Furthermore, while phosphorus homeostasis is tightly and homeostatically regulated by renal and hormonal mechanisms, the lack of biochemical assessment of phosphorus status (such as serum or urinary phosphorus) limits the ability to confirm whether the intervention effectively altered phosphorus availability. Although serum phosphate concentrations may not exhibit measurable fluctuations following short-term supplementation due to compensatory regulation, such measurements would have nonetheless provided valuable context for interpreting the observed metabolic outcomes.

Finally, both studies relied on subjective appetite assessments (VAS and paper-based questionnaires). While these tools are well-validated in appetite research and provide valuable insights into appetite regulation, self-reported measures introduce the possibility of bias. The inclusion of objective appetite markers (e.g., ghrelin and leptin levels) would have strengthened the findings.

3.7 Conclusion

This dual study demonstrated that dietary phosphorus, both in the form of potassium phosphate (Experiment 1) and naturally occurring dairy-derived phosphorus (Experiment 2), positively influenced postprandial glucose metabolism and appetite regulation. Although direct effects on core body temperature were not observed in either experiment, it is conceivable that phosphorus may influence thermogenesis without eliciting measurable alterations in core body temperature, especially considering its significant effect on appetite regulation.

Guided by the thermostatic theory, which posits that food intake is modulated by heat generated during ATP hydrolysis, phosphorus may have enhanced thermogenesis through alternative mechanisms, including increasing ATP availability, mitochondrial efficiency, and hepatic ATP production, without necessarily elevating whole-body temperature, as its effects appear to be primarily mediated at the cellular level. The use of more sensitive tools in future research, such as direct calorimetry, could provide a more precise measurement of thermogenesis and better identify the role of phosphorus in modulating thermogenic processes.

Despite these insights into thermogenesis, the study provides valuable insights into the independent role of phosphorus in glucose metabolism and

highlights the need for further research with larger populations. Findings of this study suggest that maintaining adequate dietary phosphorus intake may help mitigate glycemic surges, which are recognized risk factors for the development of insulin resistance and type 2 diabetes. This highlights the potential relevance of phosphorus in dietary strategies aimed at improving glycemic control. Such considerations could refine future dietary guidelines, particularly for populations at increased risk of impaired glucose tolerance or metabolic syndrome, where optimizing phosphorus intake may contribute to reducing the long-term risk of metabolic disorders.

Furthermore, investigating the long-term impact of phosphorus intake on postprandial metabolic responses, alongside distinguishing between the physiological impacts of organic and inorganic phosphorus sources, is crucial for gaining a more nuanced and comprehensive understanding of its role in energy metabolism and long-term metabolic health.

Chapter 4. Association of Dietary Phosphorus with Metabolic Syndrome and its Components: a Cross-Sectional Analysis of the UK National Diet and Nutrition Survey (NDNS)

4.1 Introduction

Dietary phosphorus is an essential nutrient that plays a central role in energy metabolism, glycemic regulation, and lipid homeostasis, largely through its involvement in ATP production and intracellular signaling pathways. While experimental studies have demonstrated the acute metabolic effects of phosphorus on postprandial glucose, lipid handling, and thermogenesis, population-level evidence regarding habitual phosphorus intake and its association with metabolic syndrome remains limited. This chapter examines the relationship between dietary phosphorus intake and metabolic syndrome using data from the UK National Diet and Nutrition Survey (NDNS). Building on the general background provided in Chapter 1 and the focused analyses in Chapters 2 and 3, this analysis aims to evaluate whether dietary phosphorus intake is associated with key metabolic indicators, including glycemia, blood pressure, lipid profiles, and the overall occurrence of metabolic syndrome, while providing real-world insights into population-wide phosphorus intake patterns.

4.2 Background and rational

Metabolic syndrome (MetS) is defined as the coexistence of three or more of the following interconnected health conditions: hypertension, impaired glucose tolerance, abdominal obesity, and dyslipidemia characterized by hypertriglyceridemia and decreased high-density lipoprotein cholesterol

(HDL) (Patti et al, 2018). It has been well established that individuals diagnosed with MetS are at an elevated risk for developing cardiovascular events, thereby classifying MetS as a major and global public health challenge (Kalaitzidis et al, 2005). The components of MetS are sequentially interconnected to each other, with insulin resistance being of utmost importance (Kalaitzidis et al, 2005). In short, abdominal adiposity leads to insulin resistance, which contributes to increases in triglycerides and low-density lipoprotein (LDL) levels, and decreases in HDL cholesterol levels (Patti et al, 2018). Correspondingly, hyperinsulinemia secondary to insulin resistance leads to excessive sodium retention and thus elevations in blood pressure (Brosolo et al, 2023). Because of its significant role in energy and carbohydrate metabolism, it has been proposed that any compromise in phosphate status may result in impaired glucose utilisation, insulin resistance, and hyperinsulinemia, which may subsequently lead to the onset of other co-related components of MetS (Kalaitzidis et al, 2005; Haglin, 2001). Research investigating the effects of phosphorus on MetS has yielded inconsistent findings, with studies reporting positive (Park & Han, 2021; Osadnik et al, 2020), negative (Shimodaira et al, 2017; Stoian & Stoica, 2014) and negligible associations (Terzi et al, 2015). Although the majority of studies suggest an inverse relationship (Grima et al, 2012; Ghanei et al, 2015; Gudmundsdottir et al, 2008; Vyssoulis et al, 2010), the overall evidence remains inconclusive. Consequently, a more comprehensive evaluation of the role of phosphorus in the individual components of MetS is essential to advancing knowledge in this area.

It is important to note that the existing literature, particularly cross-sectional studies, has predominantly explored the association between serum phosphate levels and MetS. Given that the NDNS dataset does not include serum phosphate measurements, the present analysis will focus on

evaluating the relationship between dietary phosphorus intake and the individual components of MetS, through a cross-sectional analysis of the NDNS Rolling Programme. Understanding these relationships could provide insight into the potential role of dietary phosphorus in metabolic health and its implications for disease prevention.

4.3 Objectives

Building on the rationale outlined above, this cross-sectional analysis aimed to systematically examine the relationship between dietary phosphorus intake and metabolic health, using data from the NDNS Rolling Programme, years 2008 to 2018. The specific objectives of this analysis are outlined in **Table 4.1**, providing a clear framework for assessing the relationship between dietary phosphorus consumption and MetS indicators within the UK adult population.

Table 4.1. Objectives and key variables for the cross-sectional analysis of dietary phosphorus intake and metabolic syndrome in the UK population.

OBJECTIVES	KEY VARIABLES/OUTCOMES
1. Analysis of P intake patterns and dietary sources in the UK population:	
To assess the distribution of daily P consumption among the UK adult population. To detect changes in estimated P intake in the UK population over time. To assess the contribution of major food groups to total P intake.	– Daily P intake distribution. – Prevalence of insufficient or excessive P intake across different age and sex categories. – Time-trend analysis of P intake from years 2008 to 2018. – Ranking of major dietary sources of P.
2. Impact of dietary P intake on MetS occurrence:	
To examine the association between dietary P intake and the occurrence of MetS. To calculate the odds ratio for the association between different daily P intake levels and the occurrence of MetS.	– Prevalence of MetS among the study population. – Odds ratio estimation for MetS, with and without adjustments for confounders.
3. Association between dietary P intake and individual components of MetS:	
To examine the association with glycemic status.	– Blood glucose levels – Glycated hemoglobin (HbA1c)
To assess the relationship with blood pressure.	– Systolic and diastolic blood pressure
To evaluate the association with abdominal.	– Waist circumference
To assess the relationship with lipid profile.	– Triglycerides – HDL cholesterol

P, phosphorus; UK, United Kingdom; MetS, metabolic syndrome; HDL, high-density lipoprotein.

4.4 Methodology

4.4.1 Data source

The National Diet and Nutrition Survey (NDNS) Rolling Programme is a continuous cross-sectional survey designed to examine the dietary habits, food consumption, nutrient intakes and nutritional status of people aged 1.5 years and older, living in private households in the United Kingdom. The survey, undertaken between years 2008 and 2018, is carried out in all four countries of the UK (England, Northern Ireland, Wales and Scotland), and is designed to be representative of the UK population. Pregnant and breastfeeding women were excluded from the survey due to their special nutritional needs. The survey includes a one-to-one interview, a 4-day estimated food diary, physical (anthropometric) and biochemical (blood and urine) measurements.

4.4.2 Ethical considerations

Written informed consent was obtained from all NDNS participants (Public Health England, 2018). The NDNS was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were granted ethical approval by the Cambridge South NRES Committee (reference no. 13/EE/0016). For ease of reference, all NDNS datasets can be accessed online through the UK Data Service: (https://www.ukdataservice.ac.uk). Since this study is a secondary analysis of the NDNS data, additional ethical approval was not required.

4.4.3 Study design and population

This study is a secondary cross-sectional analysis of the NDNS dataset. Data from years 1 to 10 (2008 to 2018) were utilized for individuals aged 19 years and older. For the first objective, which aimed to assess phosphorus intake patterns and dietary sources in the UK population, all individuals with available dietary phosphorus intake data were included, without any exclusions. This approach allowed for a comprehensive analysis of phosphorus consumption trends across different demographic groups, dietary patterns, and age categories. For the second and third objectives, which examined the association between phosphorus intake and the occurrence of MetS, as well as its individual components, a two-level inclusion process was implemented. The first level involved the exclusion of extreme outliers based on predefined criteria, while the second level accounted for missing data specific to each MetS component. These inclusion and exclusion criteria were applied to ensure data quality, minimize potential confounders, and improve the validity of the analysis. A visual representation of the inclusion process is illustrated in **Figure 4.1.**

At the first level of exclusion, individuals who identified as vegan were excluded from this analysis, due to their reliance on plant-based phosphorus sources, which are often bound to phytate. Phytate, a naturally occurring compound in plant foods, forms insoluble complexes with phosphorus, thus reducing its bioavailability. As the study aimed to investigate the effects of dietary phosphorus intake on metabolic outcomes, including participants with limited phosphorus absorption would introduce potential confounding factors. Vegetarians who consumed dairy and eggs were retained, as these are significant sources of bioavailable phosphorus, while those who exclusively consumed plant-based dairy alternatives were excluded.

To minimize errors related to extreme anthropometric measurements, individuals with an implausible BMI (less than 16 or more than 50 kg/m^2) were also excluded, as such extremes may reflect inaccuracies, underlying health conditions, or atypical metabolic profiles that could affect the validity of the analysis. Lastly, reported energy intake was screened for physiologically implausible values; females reporting an intake below 500 kcal or above 3,500 kcal, as well as males with an intake below 800 kcal or above 4,200 kcal were excluded from this analysis. These thresholds, derived from previous studies and dietary guidelines, are commonly used to identify misreporting and exclude extreme outliers, ensuring realistic dietary intake values are retained (Willet, 2013; Michels et al, 2000; Schulze et al, 2004; Fung et al, 2009; Salmeron et al, 2001; Turner-McGrievy et al, 2014). Although these cut-offs address implausible reporting, underreporting within plausible intake ranges could not be fully detected due to the lack of detailed physiological data required for calculating the ratio of reported energy intake to estimated energy requirements (EI:EER). Consequently, some residual bias related to energy misreporting may persist, which is a well-documented limitation of self-reported dietary surveys (Livingstone & Black, 2003; Garriguet, 2008).

At the second level of exclusion, additional filtering was applied based on data availability. When assessing MetS occurrence, participants with missing data for any of the following biomarkers –glucose, HbA1c, SBP, DBP, triglycerides, waist circumference, or HDL cholesterol– were excluded from the analysis. When assessing individual components of MetS, however, exclusion criteria were applied separately for each component based on data availability for that specific marker. For example, in the analysis of phosphorus intake and waist circumference, only

participants with recorded waist circumference measurements were excluded. Similarly, for analyses involving blood pressure, only individuals with available systolic and diastolic blood pressure data were considered. This stepwise approach maximizes the sample size for each analysis while maintaining data integrity and analytical consistency.

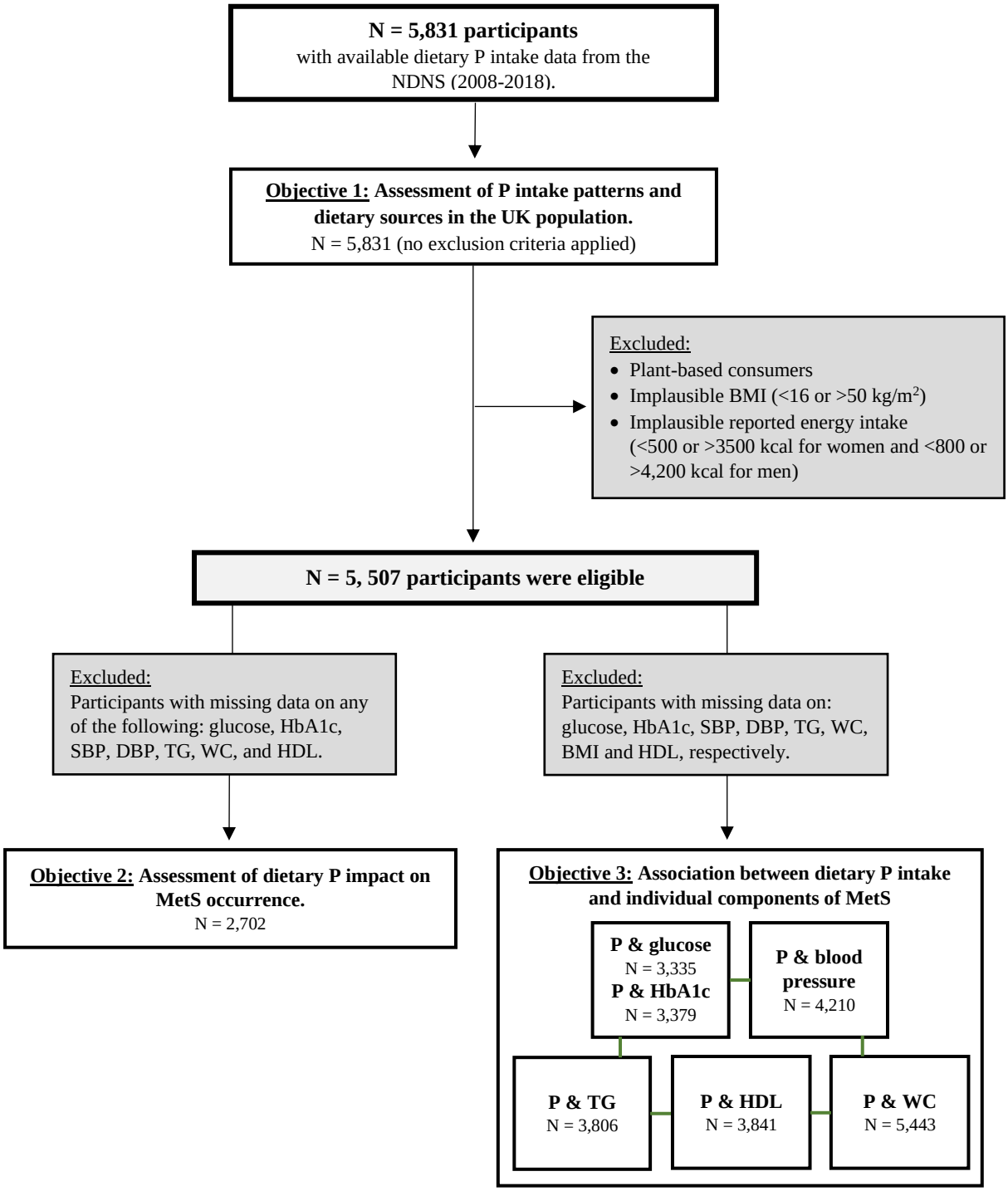


Figure 4.1. Flowchart of exclusion criteria and study population selection for each analysis.

P, phosphorus; NDNS, National Diet and Nutritional Survey; UK, United Kingdom; BMI, body mass index; HbA1c, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; WC, waist circumference; BMI, body mass index; HDL, high-density lipoprotein; MetS, metabolic syndrome.

4.4.4 Dietary assessment

NDNS participants were asked to retain a record of everything eaten and drunk over a period of four consecutive days. Estimations of serving and portion sizes were self-described using standard household measures and/or weights from nutritional labels. Records were then reviewed by trained assessors during face-to-face visits, to ensure validity and precision of all the self-reported dietary information.

4.4.5 Health markers

Blood sampling took place two to four months after the 4-day food diary was completed and was carried out by qualified nurses. Blood withdrawal followed an overnight fast for all adults, except for individuals with diabetes or those who declined to fast. The latter provided non-fasting blood samples instead. Blood pressure was recorded using an Omron-HEM907 automated validated monitor. Insights on glycemic status were inferred from glucose and glycated hemoglobin (HbA1c) measured in blood samples. The lipid profile was assessed using HDL cholesterol, LDL cholesterol, and triglyceride levels. Anthropometric measurements (weight, waist circumference, and height) were taken using a weight scale, a tape measure, and a portable stadiometer.

4.4.6 Phosphorus intake

Macro and micronutrient intake levels, including that of phosphorus, were available in the NDNS database and were deduced from participants' food records using DINO (Diet In Nutrients Out), an integrated dietary recording and analysis system, and the UK Nutrient Databank. The UK Nutrient Databank is generated in accordance with food tables from 'McCance and Widdowson's Composition of Foods', the Food Standards Agency's food

portion sizes, and data from manufacturers. Data on the average daily phosphorus consumption (mg/day) was calculated from both plant and animal phosphorus sources, in addition to phosphorus-containing supplements. All foods listed in the NDNS dataset were categorized into major food groups. Each food group's contribution to daily phosphorus intake per individual was calculated separately. The contribution was then summed across the study population, divided over the sum of daily phosphorus intake, and grouped by gender.

4.4.7 Classification of subjects with metabolic syndrome

According to the National Cholesterol Education Program Expert Panel (NCEP) and Adult Treatment Panel III (ATP III) criteria, an individual is classified as having MetS if they meet at least three of the following criteria: 1) elevated waist circumference: 88 cm or more for women, and 102 cm or more for men; 2) elevated blood pressure: 130 mm Hg or more for systolic blood pressure (SBP), or 85 mm Hg or more for diastolic blood pressure (DBP), or taking any antihypertensive drug treatments; 3) elevated triglyceride level: 150 mg/dl or more; 4) reduced HDL cholesterol level: 50 mg/dl or less for women, and 40 mg/dl or less for men; 5) elevated fasting blood sugar level: above 5.55 mmol/L, or taking any glucose-lowering medications (National Cholesterol Education Program, 2002).

4.4.8 Covariates

Several covariates were included in the analysis to account for potential confounding factors that could influence the association between phosphorus intake and MetS. These covariates were selected based on their established relevance in the literature and their potential impact on both phosphorus metabolism and MetS components. Demographic variables

included age and sex (male/female) to control for age-related metabolic variations and sex differences in dietary intake and MetS prevalence. The anthropometric variables included weight, height, and body mass index (BMI). To account for individual variations in habitual energy intake and minimize the impact of misreporting, energy density adjustments were applied by calculating phosphorus intake per 1000 kcal, through the ratio of daily phosphorus intake to total energy intake.

Lifestyle factors including smoking status (smoker or non-smoker) and alcohol consumption (drinker or non-drinker) were also considered. The inclusion of these factors helped account for their potential effects on MetS risk and dietary habits, ensuring that the observed associations between phosphorus intake and MetS components are not driven by confounding variables but reflect independent relationships within the study population.

4.4.9 Statistical analysis

Analyses were performed in SPSS Statistics (version 26) and R (version 4.2.2). Categorical data was presented as N (%). Continuous data was presented either as mean values with standard deviation or as median with 25th to 75th percentiles. Logistic regression models were used to examine the association between phosphorus intake and the risk of MetS. MetS was treated as a binary outcome variable (0 = no MetS, 1 = with MetS). The analyses were conducted using Generalized Linear Models (GLM) with a binomial family and the logit link function. The statistical models were adjusted in a stepwise manner; incorporating key demographic and lifestyle factors based on their potential relevance to MetS risk and findings from previous studies on metabolic health. To assess the effect of phosphorus intake, daily total phosphorus intake was categorized into five levels

(quintiles). For all models, the significance of individual predictors was evaluated using Wald tests. The odds ratios (OR) and their 95% confidence intervals (CI) were computed for each predictor to quantify the magnitude of associations. Variance inflation factors (VIF) were assessed to check for multicollinearity among the predictors. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test, with a non-significant p-value suggesting good model fit.

Kruskal-Wallis test was used to assess differences in each MetS variable across phosphorus intake quintiles, as the data did not meet normality assumptions. If the Kruskal-Wallis test was significant, pairwise Wilcoxon rank-sum tests with Bonferroni adjustment were performed to identify which groups differed. To adjust for potential confounders, analysis of covariance (ANCOVA) was performed using the aov() function. Type II ANOVA was conducted to further assess the significance of each predictor. Pairwise comparisons of estimated marginal means (EMMs) were conducted using the emmeans package, which adjusts for covariates and provides a clearer interpretation of group differences. Bonferroni correction was applied to account for multiple comparisons in post-hoc tests. P values of less than or equal to 0.05 were considered statistically significant throughout the study.

4.5 Results

4.5.1 Dietary phosphorus intake patterns across the population

The distribution of daily phosphorus intake across the whole population is presented in **Tables 4.2**. The median dietary phosphorus intake for the entire population was found to be 1,193 mg/day (25–75th percentiles: 961–1,447 mg). Males consumed more dietary phosphorus than females (1,345 mg/d (25–75th percentiles: 1,097–1,610 mg) versus 1,100 mg/day (25–75th percentiles: 892–1,322 mg), respectively; $p < 0.0001$). Age significantly influenced phosphorus intake among both males and females ($p < 0.0001$). Among males, phosphorus intake began to decrease for the 40-49 years group, onwards. Among females, there was an increasing trend in phosphorus intake with age which declined for the 70-79 years group ($p < 0.0001$).

4.5.2 Time-trend analysis of dietary phosphorus

There was a gender specific relationship between phosphorus intake levels observed across the survey years studied. Dietary phosphorus intake among males decreased across the survey years, from 1500 mg/day in year 1 (2008) to 1320 mg/day in year 10 (2018). In contrast, phosphorus intake levels among females remained relatively stable throughout the survey period, with no significant increases or decreases observed. **Figure 4.2** illustrates the time-trend analysis of dietary phosphorus intake among UK adult, stratified by gender, across the survey years 2008 to 2018.

Table 4.2. Daily phosphorus and energy intake stratified by sex and age groups.

			Median (25th–75th percentiles) across sex				Median (25th–75th percentiles) across age groups (years)														
N = 5,831							19–29		30–39		40–49		50–59		60–69		70–79		≥ 80		
Marker	**Median**	**IQR**	**Sex**	**Median**	**IQR**	***p*-value**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	***p*-value**
P intake (mg per day)	1,193	961-1,447	**M**	1,345	1,097-1,610	**<0.0001***	1,364	1,098-1,641	1,384	1,172-1,652	1,358	1,106-1,643	1,364	1,106-1,635	1,342	1,099-1,557	1,292	1,049-1,490	1,178	965-1,370	**<0.0001***
			F	1,100	892-1,322		1,029	826-1,256	1,099	878-1,312	1,100	902-1,339	1,134	930-1,355	1,141	933-1,341	1,104	908-1,306	977	792-1,219	**<0.0001***
P intake (mg/1000 kcal per day)	677	602-773	**M**	692	592-741	**<0.0001***	632	555-704	656	594-729	660	593-735	664	593-743	675	610-767	682	600-752	673	586-771	**<0.0001***
			F	660	609-794		644	565-730	665	601-760	681	600-785	716	623-814	734	647-824	734	644-849	703	622-792	**<0.0001***
Energy intake (kcal per day)	1,726	1,399-2,105	**M**	2,020	1,654-2,406	**<0.0001***	2,175	1,802-2,540	2,102	1,776-2,514	2,100	1,674-2,498	2,018	1,681-2,405	1,973	1,614-2,332	1,847	1,611-2,218	1,729	1,487-1,991	**<0.0001***
			F	1,565	1,285-1,856		1,584	1,275-1,918	1,629	1,304-1,946	1,587	1,296-1,946	1,582	1,324-1,836	1,529	1,281-1,798	1,517	1,254-1,781	1,460	1,184-1,675	**<0.0001***

IQR, interquartile range; P, phosphorus; M, males; F, females; kcal, kilocalories.
Kruskal–Wallis H-test was used to assess differences. * P-value <0.05 denoted statistical significance.

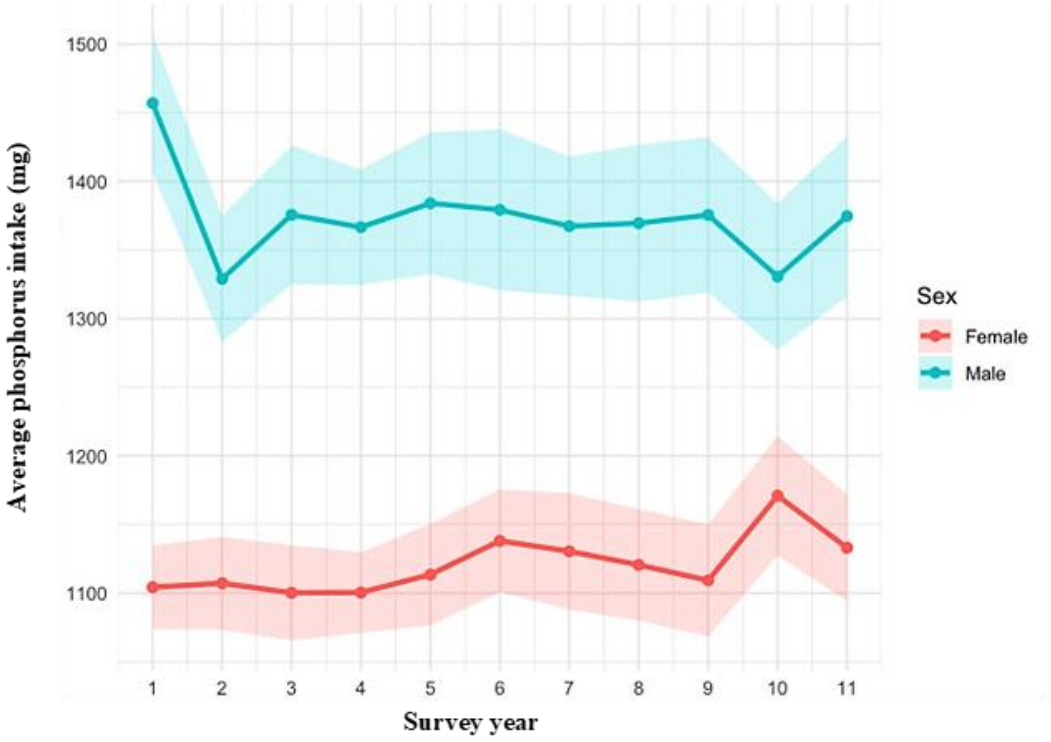


Figure 4.2. Average phosphorus intake (mg) among UK adults (19+ years), stratified by sex, across survey years 2008-2018.

Shaded areas represent the 95% confidence internal around the mean.

4.5.3 Contribution of major food groups

All foods listed in the NDNS dataset were categorized into major food groups. Each food group's contribution to daily phosphorus intake per individual was calculated separately. The contribution was then summed across the study population, divided over the sum of daily phosphorus intake, and grouped by gender. The contribution of major food groups to the daily intake of phosphorus (stratified by gender) is shown in **Figure 4.3**. Animal-based foods were the highest contributors to daily phosphorus intake. Among males, the 'meat, poultry, fish, and eggs' subgroup was the main contributor to total phosphors intake (27.16%), followed by the 'milk and dairy' subgroup (21.51%). Alternatively, among females, the 'milk and dairy' subgroup was the main contributor to total phosphors intake (25.05%), followed by the 'meat, poultry, fish, and eggs' subgroup (24.42%). 'Fats and oils', 'fruits', and 'dietary supplements' were the lowest contributors to daily phosphorus intake in both males and females. Pairwise comparisons of mean phosphorus intake showed significant differences between each of the following main food groups, in both males and females: (i) meat, poultry, fish, and eggs; (ii) milk and dairy; (iii) bread, grains, and cereal products; (iv) vegetables; and (v) beverages, with the exception of mean phosphorus intake from (meat, poultry, fish, and eggs) and (milk and dairy) food groups among females, which appeared to be rather equivalent (**Supplementary table 2**).

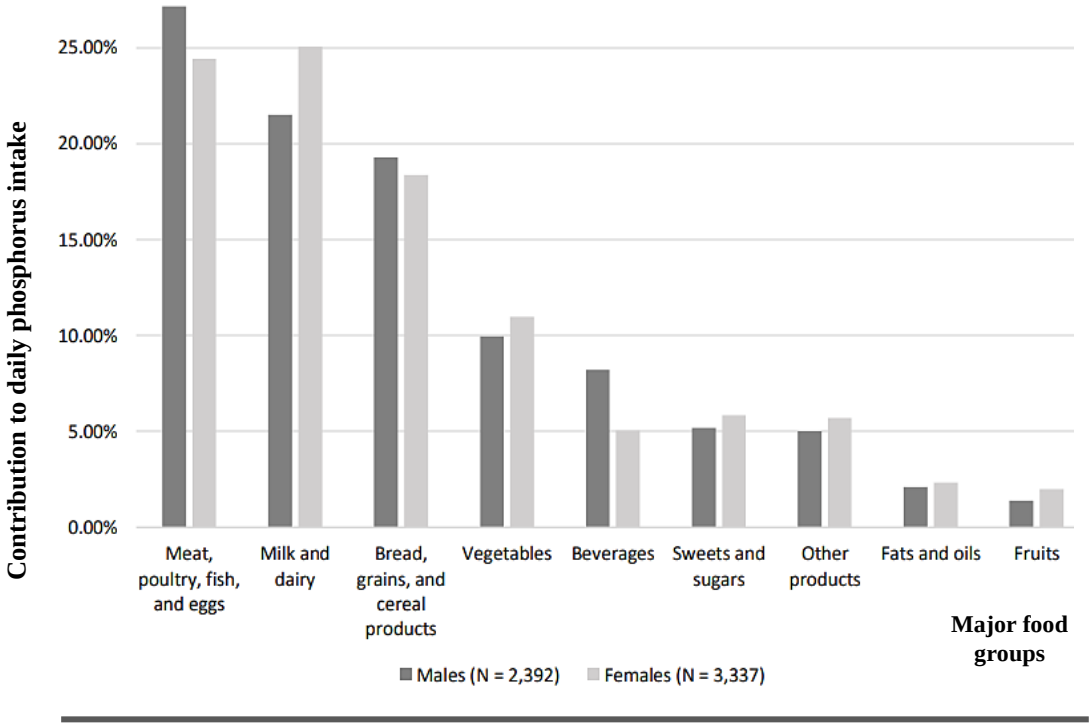


Figure 4.3. Contribution of major food groups to daily phosphorus

4.5.4 Metabolic syndrome occurrence in the UK population

This analysis included 2,702 participants, with individuals lacking records for weight, height, waist circumference, blood pressure, triglycerides, HDL cholesterol, or glucose being excluded. Over the survey years 2008 to 2018, 681 participants (25.2%) met at least three criteria for MetS and were classified as having the condition.

Sex, age, BMI, and alcohol consumption were significantly associated with MetS, as outlined in **Table 4.3**. Among those with MetS, 48% were males. The condition was most prevalent in those aged 60-69 years, and the highest number of cases was observed in the obese category (BMI 30–39.9 kg/m^2). Smoking status was not significantly associated with MetS, as 85% of affected individuals (577 cases) were non-smokers. However, alcohol consumption showed a significant association, with 63% of those with MetS reporting alcohol intake.

When examining phosphorus intake across quintiles, MetS cases were distributed relatively evenly, with no clear trend observed. The number of cases ranged from 129 to 146 per quintile **(Table 4.3**). Similarly, daily phosphorus intake and phosphorus density did not differ significantly between individuals with and without MetS.

In addition, fasting glucose, HbA1C, SBP, DBP, triglycerides, HDL cholesterol, and waist circumference were all significantly different between individuals with and without MetS.

Table 4.3. Prevalence of metabolic syndrome and its association with key variables.

Variables N = 2,702	No MetS (74.8%) N = 2,021	MetS (25.2%) N = 681	p-value
Sex			
Males	786 (70.6%)	328 (29.4%)	<0.0001*
Females	1,235 (77.8%)	353 (22.2%)	
Age (years)			
19-29	275 (94.2%)	17 (5.8%)	
30-39	367 (84.6%)	67 (15.4%)	
40-49	452 (77.5%)	131 (22.5%)	
50-59	363 (68%)	171 (32%)	<0.0001*
60-69	298 (63.1%)	174 (36.9%)	
70-79	183 (67.5%)	88 (32.5%)	
>79	83 (71.6%)	33 (28.4%)	
BMI category (kg/m^2)			
<18.5	19 (100%)	0 (0%)	
18.5-24.9	838 (95.9%)	36 (4.1%)	
25.0-29.9	787 (74.5%)	270 (25.5%)	<0.0001*
30.0-39.9	353 (51.6%)	331 (48.4%)	
>40.0	24 (35.3%)	44 (64.7%)	
Smoking status			
Non-smoker	1761 (75.3%)	577 (24.7%)	0.112
Smoker	260 (71.4%	104 (28.6%)	
Drinking status			
Non-drinker	707 (71.1%)	287 (28%)	<0.0001*
Drinker	1,314 (76.9%)	394 (23.1)	
Dietary P intake			
Daily P intake (mg/day)	1,248 (371)	1,222 (363)	0.217
P density (mg/1000 kcal)	705 (146)	701 (153)	0.595
Daily P intake quintiles (mg/day)			
<915	356 (71.1%)	145 (28.9%)	
915 – 1,106	413 (76.2%)	129 (23.8%)	
1,107 – 1,294	413 (76.2%)	129 (23.8%)	0.0732
1,295 – 1,509	385 (72.5%)	146 (27.5%)	
>1,509	454 (77.5%)	132 (22.5%)	
Fasting blood glucose (mg/dl)	92.3 (16.0)	111.0 (30.0)	<0.0001*
Glycated hemoglobin, HbA1c (%)	5.5 (0.6)	6.0 (1.0)	<0.0001*
Systolic blood pressure (mmHg)	122.4 (15.7)	136.4 (17.0)	<0.0001*
Diastolic blood pressure (mmHg)	72.3 (10.4)	79.9 (11.3)	<0.0001*
Triglycerides (mg/dl)	90.5 (46.1)	179.3 (88.8)	<0.0001*
HDL cholesterol (mg/dl)	60.8 (17.0)	45.1 (13.6)	<0.0001*
Waist circumference (cm)	89.2 (12.8)	104.6 (12.1)	<0.0001*

MetS, metabolic syndrome; BMI, body mass index; P, phosphorus; HDL, high density lipoprotein.
Categorical variables are presented as weighted percentage, while continuous data are presented as median (interquartile range).
*P-values <0.05 denoted statistical significance.

4.5.5 Dietary phosphorus and metabolic syndrome risk

Logistic regression analysis was employed to examine the association between dietary phosphorus intake, categorized by quintiles, and the risk of MetS. Three models were used to assess this relationship: the crude/unadjusted model, Model 1 (adjusted for age, sex and BMI), and Model 2 (adjusted for age, sex, BMI, daily energy intake, smoking, and drinking status) (**Table 4.4**).

In the crude model, the odds ratios (OR) for quintile 2, quintile 3, and quintile 4 were 0.77, 0.77, and 0.93, respectively, with only quintile 5 exhibiting a statistically significant reduction in the odds of MetS, as outlined in **Table 4.4**. The OR for quintile 5 was 0.71 (95% CI: 0.54, 0.94, p = 0.016), indicating a potential protective effect of higher phosphorus intake, with individuals in quintile 5 (with the highest phosphorus intake) having a 29% lower risk of MetS compared to those in quintile 1 (the reference group).

In Model 1, adjusting for age, sex, and BMI, the odds ratios for quintiles 2, 3, 4, and 5 were 0.67 (p = 0.013), 0.64 (p = 0.005), 0.77 (p = 0.09), and 0.55 (p = 0.0003), respectively. These findings indicate that after accounting for age, sex, and BMI, individuals in quintiles 2, 3, and 5 continued to show significant reductions in the odds of MetS, particularly in quintile 5, where the OR was 0.55 (95% CI: 0.39, 0.76). This corresponds to a 45% lower risk of MetS compared to quintile 1 and was highly significant (p = 0.0003).

In Model 2, which additionally adjusted for daily energy intake, smoking, and drinking status, the association between phosphorus intake and MetS remained robust. The OR for quintile 2 was 0.66 (p = 0.014), for quintile 3 was 0.59 (p = 0.002), and for quintile 5 was 0.44 (p = 0.0004), suggesting a

highly significant inverse association between phosphorus intake and MetS risk (**Table 4.4**). Specifically, quintile 5, with the highest phosphorus intake, had a 56% lower risk of MetS compared to quintile 1.

In summary, the results from the logistic regression models demonstrate a consistent inverse association between higher phosphorus intake and a reduced risk of MetS across all models.

Table 4.4. Logistic regression analysis of the association between phosphorus intake and metabolic syndrome risk.

Phosphorus intake (mg/d)	N (=2,702)	Crude OR (95 CI %)	*p*-value	Model 1 OR (95 CI %)	*p*-value	Model 2 OR (95 CI %)	*p*-value
QUINTILE 1 (<915)	501	*Reference*	-	*Reference*	-	*Reference*	-
QUINTILE 2 (915–1,106)	542	0.77 (0.58, 1.01)	0.059	0.67 (0.48, 0.92)	0.013*	0.66 (0.47, 0.91)	0.014*
QUINTILE 3 (1,107–1,294)	542	0.77 (0.58, 1.01)	0.059	0.64 (0.46, 0.87)	0.005**	0.59 (0.41, 0.82)	0.002**
QUINTILE 4 (1,295–1,509)	531	0.93 (0.71, 1.22)	0.605	0.77 (0.56, 1.04)	0.096	0.70 (0.48, 1.01)	0.059
QUINTILE 5 (>1,509)	586	0.71 (0.54, 0.94)	0.016*	0.55 (0.39, 0.76)	0.0003***	0.44 (0.28, 0.69)	0.0004***

OR, odds ratio; CI, confidence interval.
Model 1 adjustments: Age, sex, and body mass index (BMI).
Model 2 adjustments: Age, sex, body mass index (BMI), daily energy intake, smoking and drinking status.
P-values < 0.05 denote statistical significance. Asterisks indicate significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

4.5.6 Dietary phosphorus and components of metabolic syndrome

The association between dietary phosphorus and the individual components of MetS was evaluated using two measures: total phosphorus intake and total energy-adjusted phosphorus intake (phosphorus density), defined as milligrams of phosphorus intake per 1,000 kilocalories. Due to variations in data availability, the sample sizes for each metabolic component differed, with the corresponding numbers detailed in **Table 4.6**. This approach ensured the optimal use of available data while minimizing data loss.

4.5.6.1 Total phosphorus intake and metabolic syndrome components

The Pearson correlation coefficients between total phosphorus intake levels (mg/day) and components of MetS are presented in **Table 4.5**.

Total phosphorus intake was strongly and positively correlated with daily energy intake ($r = 0.784$, $p < 0.001$), potassium ($r = 0.815$, $p < 0.001$), calcium ($r = 0.735$, $p < 0.001$), and sodium intake ($r = 0.614$, $p < 0.001$), indicating that individuals with higher phosphorus intake consumed larger quantities of food overall. Weak but significant positive correlations were observed with weight ($r = 0.166$, $p < 0.001$), height ($r = 0.354$, $p < 0.001$), waist circumference ($r = 0.076$, $p < 0.001$), and SBP ($r = 0.052$, $p = 0.001$). No significant association was observed with BMI ($r = -0.021$, $p = 0.123$). Total phosphorus intake was largely unrelated to glycemic and lipid markers, including HbA1c, fasting glucose, triglycerides, and LDL cholesterol. Minor negative correlations were observed with HDL cholesterol ($r = -0.041$, $p = 0.010$), total cholesterol ($r = -0.052$, $p = 0.006$), and MetS score ($r = -0.056$, $p = 0.003$).

The relationship between total dietary phosphorus intake and the individual components of MetS was further assessed through quintiles to elucidate potential associations. Phosphorus intake quintiles were categorized as follows: the first quintile (<915 mg/day), the second quintile (915 – 1,106 mg/day), the third quintile (1,107 – 1,294 mg/day), the fourth quintile (1,295 – 1,509 mg/day), and the fifth quintile (>1,509 mg/day). After adjusting for age, sex, and BMI, significant associations were observed between dietary phosphorus intake and several components of metabolic syndrome, namely triglycerides, HDL cholesterol, and waist circumference, as presented in **Table 4.6.** Triglyceride levels were significantly lower among participants in the second and third phosphorus intake quintiles (88.6 mg/dL, IQR = 62.0) compared with those in the lowest quintile (97.4 mg/dL, IQR = 70.9), indicating a potential non-linear, inverse relationship between phosphorus intake and circulating triglycerides. HDL cholesterol concentrations were significantly reduced in the highest phosphorus intake quintile (52.6 mg/dL, IQR = 21.3) compared with the lowest quintile (54.1 mg/dL, IQR = 22.8), suggesting that excessive phosphorus intake may be associated with modest reductions in HDL levels. In contrast, waist circumference was significantly higher in the fourth and fifth quintiles (92.8 cm, IQR = 20.3 and 93.5 cm, IQR = 19.6, respectively) relative to the lowest quintile (90.5 cm, IQR = 19.2), reflecting a positive, linear association between higher phosphorus intake and central adiposity.

4.5.6.2 Phosphorus density and metabolic syndrome components

The Pearson correlation coefficients between phosphorus density and components of MetS are outlined in **Table 4.5**. Age was weakly positively correlated with phosphorus density ($r = 0.132$, $p < 0.001$). Phosphorus

density was weakly inversely correlated with height ($r = -0.100$, $p < 0.001$) and daily energy intake ($r = -0.301$, $p < 0.001$), and weakly positively correlated with BMI ($r = 0.084$, $p < 0.001$). Positive correlations were observed with potassium ($r = 0.173$, $p < 0.001$) and calcium intake ($r = 0.229$, $p < 0.001$), suggesting that nutrient-dense diets provide more phosphorus per calorie. Most metabolic markers, including glucose, SBP, DBP, LDL, total cholesterol, and MetS score, were not significantly associated with phosphorus density, though small significant correlations were observed with HbA1c ($r = 0.049$, $p = 0.005$), triglycerides ($r = -0.049$, $p = 0.003$), and HDL cholesterol ($r = 0.052$, $p = 0.001$).

To build on these findings, the association between phosphorus density quintiles (mg/1000 kcal) and MetS components was subsequently examined (**Table 4.7**). Participants were categorized into quintiles of phosphorus density as follows: the first quintile (< 585 mg/1,000 kcal), the second quintile (585 – 647 mg/1,000 kcal), the third quintile (648 – 711 mg/1,000 kcal), the fourth quintile (712 – 798 mg/1,000 kcal), and the fifth quintile (> 799 mg/1,000 kcal).

DBP demonstrated a significant and progressive decline across phosphorus density categories, with the fifth quintile differing significantly from all lower quintiles. Median DBP values decreased from 74.5 mmHg (IQR = 15.0) in the lowest quintile to 72.5 mmHg (IQR = 13.5) in the highest, indicating a linear inverse association ($p = 0.001$). This pattern suggests that higher phosphorus density may contribute to lower diastolic pressure, independent of BMI, sex, or age.

Similarly, median triglyceride levels were significantly reduced in the highest phosphorus density quintile compared with all lower quintiles. Participants in the fifth quintile exhibited a median triglyceride

concentration of 88.6 mg/dL (IQR = 62.0), whereas the median remained relatively stable across the first four quintiles at 97.4 mg/dL (IQR = 70.9), representing a decline of approximately 9 mg/dL (p = 0.002). The consistency of triglyceride levels across the lower quintiles, followed by a notable decrease in the fifth, indicates that the beneficial effect of phosphorus on triglycerides may occur only at higher levels of intake rather than following a dose-dependent trend.

Regarding waist circumference, median values were slightly higher in the fifth quintile compared with the first, increasing from 91.4 cm (IQR = 20.8) to 92.4 cm (IQR = 20.8). Although this difference reached statistical significance (p = 0.001), the magnitude of change was minimal and unlikely to be of clinical importance.

Table 4.5. Pearson correlations of total phosphorus intake and phosphorus density with anthropometric, dietary, and metabolic parameters.

	Total phosphorus intake (mg/day)			Phosphorus density (mg/1000 kcal)		
Variable	**N**	**Pearson Correlation**	**p-value**	**N**	**Pearson Correlation**	**p-value**
Age	5,507	-0.019	0.169	5,507	**0.132****	**0.000**
Weight	5,507	**0.166****	**0.000**	5,507	0.023	0.083
Height	5,507	**0.354****	**0.000**	5,507	**-0.100****	**0.000**
Daily energy intake	5,507	**0.784****	**0.000**	5,507	**-0.301****	**0.000**
Daily Na intake	5,507	**0.614****	**0.000**	5,507	**-0.133****	**0.000**
Daily K intake	5,507	**0.815****	**0.000**	5,507	**0.173****	**0.000**
Daily Ca intake	5,507	**0.735****	**0.000**	5,507	**0.229****	**0.000**
HbA1c	3,379	0.001	0.951	3,379	**0.049****	**0.005**
Glucose	3,335	0.024	0.163	3,335	0.016	0.356
SBP	4,210	**0.052****	**0.001**	4,210	0.010	0.525
DBP	4,210	0.011	0.468	4,210	**-0.036***	**0.020**
Triglycerides	3,806	0.001	0.968	3,806	**-0.049****	**0.003**
WC	5,443	**0.076****	**0.000**	5,443	0.018	0.193
HDL	3,841	**-0.041***	**0.010**	3,841	**0.052****	**0.001**
LDL	2,694	-0.037	0.053	2,694	0.011	0.558
Total cholesterol	2,722	**-0.052****	**0.006**	2,722	0.013	0.497
MetS score	2,702	**-0.056****	**0.003**	2,702	-0.014	0.455
BMI	5,507	-0.021	0.123	5,507	**0.084****	**0.000**

Na, sodium; K, potassium; Ca, calcium, HbA1c, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; MetS, metabolic syndrome; BMI, body mass index.
**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 4.6. Associations of total daily phosphorus intake (mg/day) quintiles with individual components of metabolic syndrome.

TOTAL P INTAKE (mg/day)	**QUINTILE 1** (< 915)		**QUINTILE 2** (915 – 1,106)		**QUINTILE 3** (1,107 – 1,294)		**QUINTILE 4** (1,295 – 1,509)		**QUINTILE 5** (> 1,509)		**p-values**	
	Med	**IQR**	**Med**	**IQR**	**Med**	**IQR**	**Med**	**IQR**	**Med**	**IQR**	***p*-value†**	***p*-value‡**
N = 3,335	624		670		667		658		716			
FBG (mg/dl)	91.8	14.2	92.1	13.0	93.2	13.5	93.3	14.1	92.6	13.4	0.201	0.151
N = 3,379	634		686		683		667		709			
HbA1c (%)	5.5	0.5	5.4	0.6	5.5	0.5	5.5	0.5	5.4	0.4	0.461	0.056
N = 4,210	829		852		842		837		850			
SBP (mmHg)	122.5	24.5	123.0	24.3	124.0	22.0	124.5	21.5	125.8	19.5	**0.005**	0.415
DBP (mmHg)	73.0	14.5	73.0	14.0	73.0	15.0	73.5	15.5	73.5	14.5	0.904	0.717
N = 3,806	712		764		764		763		803			
TG (mg/dl)	97.4[a]	70.9	88.6[b]	62.0	88.6[b]	62.0	97.4	70.9	97.4	79.7	0.339	**<0.0001**
N = 3,841	721		771		771		769		809			
HDL (mg/dl)	54.1[a]	22.8	54.1	22.4	55.3	22.8	53.8	21.7	52.6[b]	21.3	**0.004**	**0.001**
N = 5,443	1,803		1,081		1,094		1,083		1,102			
WC (cm)	90.5[a]	19.2	91.4	21.9	91.7	20.4	92.8[b]	20.3	93.5[b]	19.6	**<0.0001**	**0.001**

P, phosphorus; SD, standard deviation; Med, median; IQR, interquartile range; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL, high density lipoprotein cholesterol; WC, waist circumference.

† Unadjusted p-values derived from the Kruskal-Wallis test to assess if each MetS component differs across total phosphorus quintiles.

‡ P-values adjusted for age, sex, and BMI, derived from the Kruskal-Wallis test to assess if each MetS component differs across total phosphorus quintiles.

Values with different superscripts (a, b, c) indicate statistically significant differences between phosphorus total intake quintiles based on Bonferroni-adjusted p-values from pairwise Wilcoxon rank-sum tests. Superscripts are not applied to unadjusted values. Pairwise comparisons were conducted only where the Kruskal–Wallis test indicated overall group differences. Bonferroni correction was used to adjust for multiple testing across all 10 pairwise comparisons.

Table 4.7. Associations of phosphorus density (mg/1000 kcal) quintiles with individual components of metabolic syndrome.

TOTAL P DENSITY (mg/ 1000 kcal)	**QUINTILE 1** (< 585)		**QUINTILE 2** (585 – 647)		**QUINTILE 3** (648 – 711)		**QUINTILE 4** (712 – 798)		**QUINTILE 5** (> 799)		**p-values**	
	Med	**IQR**	**Med**	**IQR**	**Med**	**IQR**	**Med**	**IQR**	**Med**	**IQR**	***p*-value†**	***p*-value‡**
N = 3,335	653		648		690		672		672			
FBG (mg/dl)	92.6	14.1	93.0	13.0	92.9	13.9	91.7	13.0	92.6	14.0	0.598	0.714
N = 3,379	677		658		699		674		671			
HbA1c (%)	5.4	0.4	5.5	0.5	5.5	0.5	5.5	0.5	5.5	0.5	0.128	0.627
N = 4,210	793		834		857		849		877			
SBP (mmHg)	123.0	23.5	123.8	22.5	124.0	22.0	124.0	22.5	124.5	23.5	0.433	0.181
DBP (mmHg)	74.5[a]	15.0	74.0[a]	15.0	73.0[a]	15.0	73.0[a]	14.0	72.5[b]	13.5	**0.008***	**0.001***
N = 3,806	749		748		776		767		766			
TG (mg/dl)	97.4[a]	70.9	97.4	79.7	97.4	70.9	97.4	62.0	88.6[b]	62.0	0.259	**0.002***
N = 3,841	756		756		783		774		772			
HDL (mg/dl)	52.6	22.4	53.2	21.1	53.0	20.9	55.1	23.6	55.7	22.4	**0.002***	0.301
N = 5,443	1,087		1,092		1,094		1,088		1,082			
WC (cm)	91.4[a]	20.8	91.4	20.5	92.2	20.0	92.1	20.1	92.4[b]	20.8	0.491	**0.001***

, phosphorus; SD, standard deviation; Med, median; IQR, interquartile range; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; SBP, systolic blood pressure; DBP, iastolic blood pressure; TG, triglycerides; HDL, high density lipoprotein cholesterol; WC, waist circumference.
Unadjusted p-values derived from the Kruskal-Wallis test to assess if each MetS component differs across phosphorus density quintiles.
P-values adjusted for age, sex, and BMI, derived from the Kruskal-Wallis test to assess if each MetS component differs across phosphorus density quintiles.
'alues with different superscripts (a, b, c) indicate statistically significant differences between phosphorus intake density quintiles based on Bonferroni-adjusted p-values from airwise Wilcoxon rank-sum tests. Superscripts are not applied to unadjusted values. Pairwise comparisons were conducted only where the Kruskal–Wallis test indicated overall roup differences. Bonferroni correction was used to adjust for multiple testing across all 10 pairwise comparisons.

4.6 Discussion

Unlike many prior studies that primarily focus on the overall prevalence of MetS and its associations with specific nutrients, this study provides a more comprehensive analysis by examining both the occurrence of MetS and its individual components. This approach offers a more nuanced understanding of the potential associations between dietary phosphorus intake and metabolic health outcomes within a nationally representative UK population.

On average, the median dietary phosphorus intake of the study population met the Reference Nutrient Intake (RNI) for healthy adults. However, notable differences were observed based on sex, with men exhibiting higher phosphorus intake levels than women. Temporal trends further revealed a decline in phosphorus intake among men, while intake remained relatively stable among women. Analysis of dietary sources indicated that the primary contributors to phosphorus intake were meat, poultry, fish, and eggs, reflecting the predominance of animal-based sources in the population's diet. Given that phosphorus is naturally abundant in these foods, as well as in dairy products and whole grains, its intake is largely influenced by dietary patterns. However, variations in intake may also be driven by differences in food processing, as phosphate additives are widely used in processed foods, potentially contributing to disparities in phosphorus consumption across population subgroups.

While previous observational studies have predominately examined the relationship between total phosphorus intake and health outcomes, this study expanded upon that approach by incorporating both total phosphorus and total energy-adjusted phosphorus intake measures (i.e. phosphorus

density), contributing to a foundational understanding of phosphorus intake within the UK population.

When evaluating the relationship between phosphorus intake and metabolic health, considering phosphorus density provides a more accurate and meaningful assessment than total phosphorus intake alone. Total phosphorus intake is inherently influenced by overall energy consumption, making it difficult to determine whether observed associations with metabolic outcomes are driven by phosphorus itself or by differences in total caloric intake. Individuals with higher energy intake naturally consume more phosphorus, which may confound the relationship between phosphorus and metabolic markers. Adjusting for energy intake allows phosphorus density to account for these variations, providing a clearer interpretation of its independent role and reducing potential confounding effects. Additionally, phosphorus density may better reflect dietary quality. Foods rich in phosphorus vary widely in their nutrient composition, and individuals consuming a diet with a higher phosphorus density may have different overall dietary patterns than those with high total phosphorus intake but lower density. This distinction is particularly relevant when examining associations with MetS, as dietary patterns that are higher in nutrient-dense foods may have different metabolic effects than those dominated by energy-dense, phosphorus-rich processed foods.

With respect to MetS risk, the logistic regression analysis demonstrated that increased phosphorus intake is associated with a significantly reduced likelihood of developing MetS. In the crude model, quintile 5 (the highest phosphorus intake group) showed a significant reduction in MetS risk, suggesting a potential protective effect of phosphorus intake at higher levels.

This association became even more pronounced after adjusting for age, sex, and BMI in Model 1, indicating a 45% lower risk of MetS for those in the highest phosphorus intake quintile compared to the reference. In Model 2, which accounted for additional factors such as daily energy intake, smoking, and drinking status, the association remained strong, with the odds ratio for quintile 5 dropping to 0.44, corresponding to a 56% lower risk of MetS for those in the highest quintile of phosphorus intake. However, the lack of significant associations in the fourth quintile in both Models 1 and 2 may point to a non-linear relationship between phosphorus intake and MetS risk, suggesting that the protective effect of phosphorus may only become apparent at higher levels of intake. Additionally, while the regression models adjusted for key factors, further research is needed to clarify whether other unmeasured confounders, such as dietary patterns or specific phosphorus sources, might influence these associations.

Findings from the current study are consistent with the growing body of evidence suggesting that phosphorus plays a key role in metabolic regulation. Although the literature offers limited research directly addressing the impact of dietary phosphorus intake on the risk of MetS occurrence, a substantial body of observational evidence indicates that serum phosphate levels are inversely correlated with MetS (Kalaitzidis et al, 2005; Vyssoulis et al, 2010; Gudmundsdottir et al, 2008; Stoian & Stoica, 2014; Ghanei et, 2005; Park et al, 2009).

Regarding the individual components of MetS, the current study revealed that the analysis of total phosphorus intake, without adjustment for energy intake, demonstrated a non-linear association with HDL cholesterol, in addition to a direct association with waist circumference. Furthermore, triglyceride levels exhibited an inverse relationship, such that the lowest total phosphorus intake quintile was associated with the highest triglyceride

concentration. Conversely, when phosphorus intake was adjusted for energy intake using phosphorus density, along with adjustments for age, sex, and BMI, notable inverse associations were observed. Higher phosphorus density was associated with significantly lower DBP and triglycerides values, with a reduction of approximately 2 mmHg and 10 mg/dl, respectively, between the first and fifth quintiles. The observed 2 mmHg reduction in DBP is not only statistically significant, but also clinically meaningful, as previous research suggests that even a modest decrease of 2mmHg in DBP at the population level could have significant public health implications (Cook et al, 1995). A reduction of this magnitude has been associated with a 17% decrease in hypertension prevalence, a 15% reduction in stroke risk, and a 6% lower risk of coronary heart disease (Cook et al, 1995), highlighting the clinical relevance of phosphorus intake in blood pressure regulation. Similarly, the 10 mg/dl decrease in triglyceride levels across phosphorus quintiles suggests a potential lipid-lowering effect of higher phosphorus intake. Reductions in triglyceride levels have been linked to improvements in cardiovascular health, as elevated triglycerides are a well-established risk factor for atherosclerosis and cardio-metabolic disease (Marston et al, 2019). These findings suggest that the effect of phosphorus on these metabolic parameters is not merely a consequence of higher caloric intake but may instead represent its independent contribution to cardio-metabolic health.

The waist circumference results from this study demonstrated a statistically significant, yet clinically negligible, increase, with only a 1 cm difference between the lowest and highest quintiles. As for glycemia, neither total phosphorus intake nor phosphorus density detected any significant associations with glucose or glycated hemoglobin levels.

Overall, the analysis of phosphorus density in this study revealed associations that were not observed when examining total phosphorus intake alone, such as the inverse association with DBP. The discrepancy in results between total phosphorus intake and phosphorus density highlights the potential for confounding in studies that do not adjust for energy intake. It also emphasizes the importance of considering phosphorus density as an independent factor in observational research, as relying solely on total phosphorus intake may lead to misleading findings, especially when energy intake is not adequately controlled for.

There are few studies in the literature that examine the relationship between dietary phosphorus intake and MetS components, as most cross-sectional research has focused on serum phosphate levels instead. This limited availability of comparable studies made it challenging to directly contextualize the present findings. However, among the available studies, several have reported results that are consistent with those observed in this study. For example, a large cohort study, including 13,400 participants, demonstrated an inverse association between phosphorus intake and blood pressure, consistent with our findings (Alonso et al, 2010). Similarly, a cross-sectional study by Elliott et al., involving 4,680 men and women, found that phosphorus intake, through diet or supplement use, had lower systolic and diastolic blood pressures in adults aged 40 years and above (Elliot et al, 2008). Additionally, a longitudinal study by Alonso et al. reported that phosphorus intake from dairy sources was associated with lower blood pressure and a reduced risk of hypertension, whereas phosphorus from inorganic additives was linked to increases in both systolic and diastolic blood pressure (Alonso et al, 2010). Results from this study are also in accordance with the Atherosclerosis Risk in Communities (ARIC) and Multi-Ethnic Study of Atherosclerosis (MESA) studies, which

found that higher phosphorus intake from dairy products, but not from dietary plant sources, was linked to lower levels of blood pressure and a lower risk of hypertension (Wu et al, 2023). Put together, these findings further support the potential role of dietary phosphorus, particularly from natural sources, in blood pressure regulation. In light of this, the International Study of Macro and Micronutrients and Blood Pressure, a cross-sectional study that included 4,680 adults from the UK, USA, Japan, and China, have reinforced an increase in phosphorus and minerals consumption levels to regulate blood pressure, as part of the recommendations for healthier eating patterns and the prevention of hypertension (Elliot et al, 2008).

Research on phosphate metabolism in dyslipidemia remains limited, with the majority of studies being conducted in animal models. While no comparable cross-sectional studies were identified in the literature, evidence from interventional studies and animal models has reported similar findings. Previous work by El Khoury et al. indicated that phosphorus enrichment and supplementation of refined bread flour, significantly reduced postprandial triglyceride levels in healthy individuals (El Khoury et al, 2020). In animals, Tanaka et al. found that phosphate restriction increased liver weight and hepatic lipid accumulation in mice, particularly when the mice were on a diet low in inorganic phosphate (Tanaka et al, 2013). Likewise, Grundman et al. observed that mice fed a high-phosphorus diet had lower triglyceride levels and non-esterified cholesterol compared to those fed an adequate phosphorus diet (Grundman et al, 2023). Another research work by Abuduli et al. reported that rats on a high-phosphate diet showed reduced visceral fat accumulation and lower non-esterified fatty acids (Abuduli et al, 2016). Despite the promising findings from various studies, the relationship between phosphate

metabolism and dyslipidemia in humans remains inconclusive, particularly as two crossover human studies found no significant associations between phosphorus intake and triglyceride levels, with no notable differences in triglycerides and total cholesterol observed between the phosphorus and non-phosphorus exposure groups (Ayoub et al, 2015; Volk et al, 2022).

Concerning glycemia, while no significant association was observed between phosphorus intake and glucose or HbA1c in this secondary analysis of the NDNS dataset, other studies have reported differing results. For example, a recent nationwide cohort study in China found a U-shaped association between dietary phosphorus intake and new-onset diabetes, with a minimal risk at 905.0 to 975.4 mg/day of phosphorus intake (Wu et al, 2023). When dietary phosphorus intake was assessed as quintiles, compared with those in the 3rd quintile (905.0 – <975.4 mg/day), significantly higher risks of new-onset diabetes were found in participants in the 1st-2nd quintiles (<905.0 mg/day: Hazard Ratio, 1.59; 95% CI, 1.30–1.94), and 4th-5th quintiles (>975.4 mg/day: Hazard Ratio, 1.46; 95% CI, 1.19–1.78) (Wu et al, 2023). Similarly, increased phosphorus consumption (>1,477 mg per day) was associated with an increased risk of type 2 diabetes in a large prospective E3N (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale) French cohort study (Mancini et al, 2018).

4.6.1 Strengths and limitations

One of the key strengths of this study is the use of data from the NDNS, which offers several advantages. The NDNS is a nationally representative survey of the UK population, encompassing a large and diverse sample. This broad representation enhances the generalizability of the findings to the wider UK population, allowing for more robust conclusions about the role of phosphorus in public health. Furthermore, the NDNS collects a wide range of health metrics, including glucose levels, blood pressure, and lipid panels, which allowed for a comprehensive analysis of the relationship between phosphorus intake and various components of MetS. By capturing these key indicators, the NDNS provides a valuable opportunity to explore how phosphorus intake might influence broader metabolic health, particularly in relation to conditions like insulin resistance, hypertension, and dyslipidemia. Another strength is that both daily phosphorus intake (mg/day) and phosphorus density (mg/1000 kcal/day) were assessed in this study. This dual approach provides a more comprehensive understanding of phosphorus consumption, as it considers not only the absolute amount of phosphorus consumed but also its distribution relative to total caloric intake. This enables a more nuanced assessment of how phosphorus intake may affect health outcomes across different dietary patterns.

However, despite these strengths, several limitations should be considered when interpreting the findings. First, the NDNS relies on self-reported dietary intake data, which introduces the possibility of recall and reporting bias, and may not fully capture daily fluctuations in phosphorus intake. This limitation is further compounded by the use of a four-day consecutive food diary, which, although widely accepted for detailed dietary assessment, may be affected by reactivity bias; participants aware of being monitored might

alter their usual eating habits, potentially impacting data accuracy. Although extreme outliers for energy intake were excluded using predefined plausibility cut-offs, underreporting within physiologically plausible ranges could not be entirely ruled out. Furthermore, the absence of detailed physiological data prevented the calculation of the energy intake to estimated energy requirement ratio (EI:EER), a method commonly used to detect energy misreporting in population-based dietary surveys (Livingstone & Black, 2003; Garriguet, 2008). Consequently, residual bias due to potential energy underreporting may persist, which could have attenuated the strength of the observed associations between dietary phosphorus and metabolic outcomes.

Additionally, a major limitation of this study is the inability to differentiate between organic phosphorus derived from natural food sources and inorganic phosphorus originating from food additives and supplements. This distinction is crucial, as inorganic phosphorus is more readily absorbed and may exert distinct metabolic effects. Furthermore, it was not possible to systematically classify and quantify phosphorus from plant-based and animal-based sources due to the broad categorization of food intake in the NDNS. The database provides food intake data at a general level, making it challenging to accurately differentiate phosphorus based on its origin. For instance, composite foods such as salads, burgers, pizzas, and mixed dishes may contain phosphorus from both animal and plant-based ingredients. This overlap in food composition prevents a precise classification of the sources of phosphorus intake. In this context, concerns have been raised about the accuracy of assessment tools used to estimate dietary phosphorus intake in large-scale studies, since it may not be clear whether total consumption levels are accounting for dietary phosphorus in all its forms, particularly

phytate-bound phosphorus and phosphorus from food additives (Gutiérrez, 2013; Calvo & Uribarri, 2013). Consequently, the EFSA Panel has highlighted the importance of developing surrogate markers for phosphorus intake that extend beyond dietary estimates, to enhance the accuracy of phosphorus consumption assessments at both individual and population levels (EFSA, 2015).

Another limitation is the inability to directly compare phosphorus intake and its effect on metabolic components between plant-based and animal-based food consumers. While such a comparison would have provided valuable insights into the metabolic implications of different dietary phosphorus sources, the number of exclusive plant-based consumers in the dataset was insufficient for meaningful comparisons. Consequently, plant-based consumers were excluded from the analysis, which was limited to animal-based food consumers, as phytate-bound phosphorus from plant-based diets is poorly absorbed.

A key methodological limitation arises from the timing of biomarker measurements. Blood sampling, including glucose, HbA1c, and lipid markers, was conducted three to four months after participants completed their four-day food diary. This lag between dietary exposure and biomarker assessment may have influenced the results, particularly for outcomes related to glycemia. Other metabolic markers, such as waist circumference, may also have been affected, as weight fluctuations over this period could impact the observed associations. Additionally, caution should be exercised when interpreting results from the NDNS dataset, as it represents free-living, largely healthy participants whose habitual dietary behaviors may differ from those of clinical or metabolically challenged populations. These characteristics, combined with the delay between dietary recording and

biomarker collection, may have attenuated observed associations, particularly for short-term biomarkers such as glucose and triglycerides.

Finally, HbA1c, which was used to assess glycemia, can be influenced by various clinical and physiological conditions. Factors such as smoking, chronic anemia, hemolysis, and severe infections can alter erythrocyte turnover, potentially altering HbA1c readings (Bonora & Tuomilehto, 2011). These conditions may compromise the accuracy of HbA1c as a marker of glycemic control. As a result, these factors introduce additional variability into the analysis and should be considered when interpreting the findings.

4.7 Conclusion

This study contributes to the growing body of evidence on the role of dietary phosphorus in metabolic health by analyzing data from the UK National Diet and Nutrition Survey (NDNS). The findings indicate that higher phosphorus intake is associated with improved metabolic markers, particularly lower diastolic blood pressure and triglyceride levels, and a reduced risk of metabolic syndrome. These results highlight the potential significance of phosphorus intake in modulating metabolic health outcomes.

However, further research is required to enhance the accuracy and interpretation of phosphorus intake assessments. Future studies should not only consider serum phosphate levels but also incorporate detailed dietary intake assessments, accounting for phosphorus sources, energy intake, and phosphorus density. Reliance on absolute phosphorus intake measures alone may introduce confounding effects, potentially leading to misinterpretation of findings. A more refined approach that considers

phosphorus density relative to total dietary intake could provide a clearer understanding of its role in metabolic regulation. Additionally, prospective studies are needed to examine the long-term effects of dietary phosphorus and to elucidate the underlying mechanisms by which phosphorus influences metabolic health. By addressing these gaps, future research can offer a more comprehensive perspective on the implications of dietary phosphorus intake and inform potential dietary guidelines for MetS prevention and management.

Chapter 5. General Discussion

This dissertation aimed to investigate the role of dietary phosphorus in postprandial metabolic responses, with a specific focus on glucose regulation and energy expenditure. To address this, three complementary approaches were employed: (1) a systematic review synthesizing existing literature on phosphorus and its metabolic effects, (2) two controlled clinical trials examining the impact of phosphorus manipulation on postprandial glycemia and thermogenesis (3) a cross-sectional analysis of the UK National Diet and Nutrition Survey (NDNS) to explore associations between habitual phosphorus intake and metabolic health markers. By integrating these methodologies, this dissertation provided a comprehensive evaluation of the metabolic implications of dietary phosphorus, offering insights into its potential role in modulating key physiological processes. A visual summary of the findings from the systematic review, experimental studies, and NDNS cross-sectional analysis is presented in **Figure 5.1**. The following discussion contextualizes the findings within the broader literature, highlighting their implications, strengths, and limitations while identifying directions for future research.

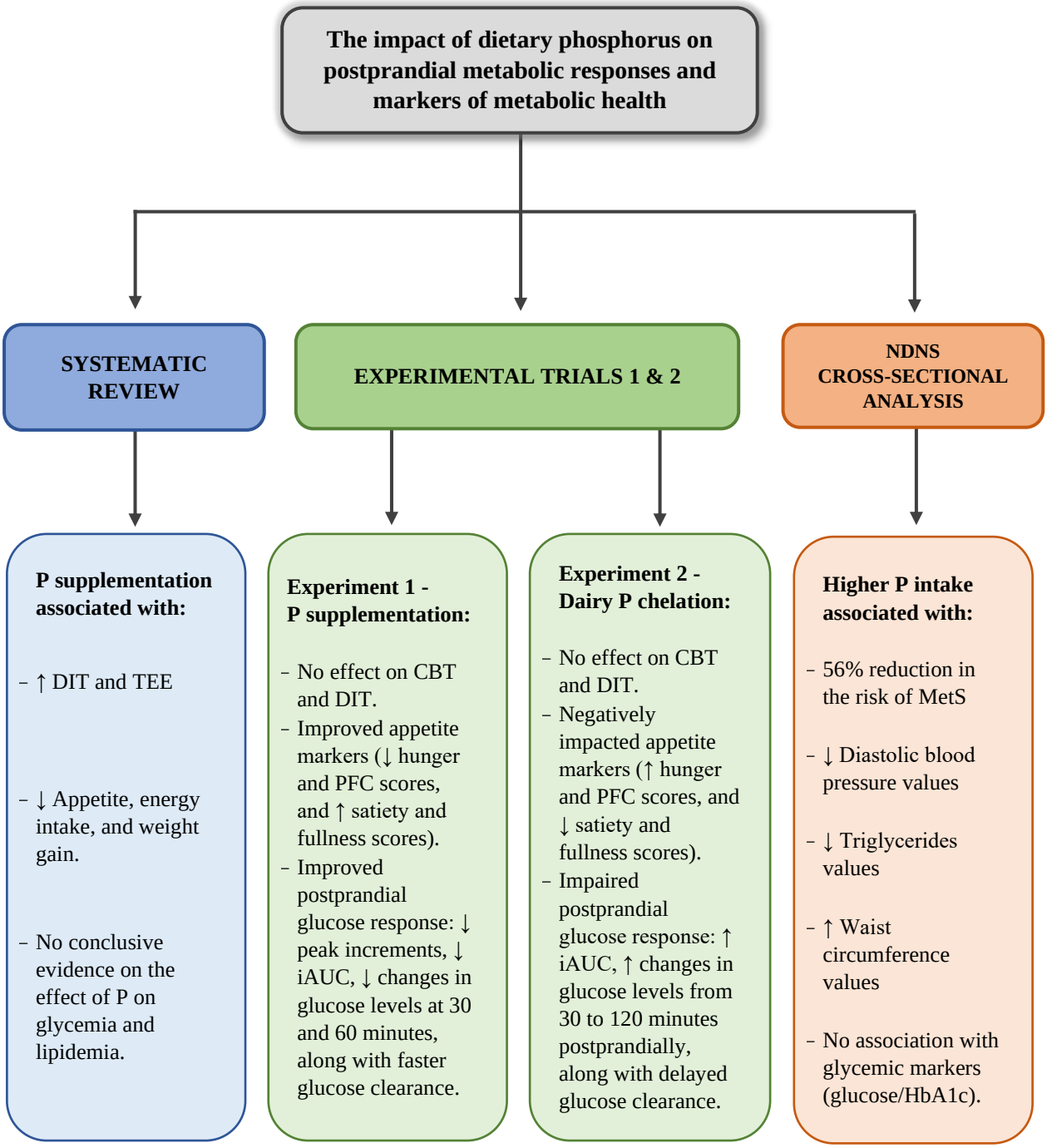


Figure 5.1. Summary of key findings: systematic review, experimental studies, and cross-sectional analysis of NDNS data.

P, phosphorus; DIT, diet induced thermogenesis; TEE, total energy expenditure; CBT, core body temperature; PFC, prospective food consumption; iAUC, incremental area under the curve, MetS, metabolic syndrome; HbA1c, glycated hemoglobin.
The arrow pointing upward (↑) indicates an increase; the arrow pointing downward (↓) indicates a decrease.

5.1 Dietary phosphorus and glycemia

5.1.1 Experimental evidence

Both clinical trials demonstrated that dietary phosphorus significantly modulates postprandial glucose regulation. In Experiment 1, inorganic phosphorus administered as potassium phosphate reduced peak glucose increments in the early postprandial phase (30–60 min), highlighting the rapid availability of inorganic phosphorus for ATP synthesis and glucose disposal. Experiment 2, using dairy-derived organic phosphorus at a higher dose, produced a more sustained reduction in glucose throughout the postprandial period. Differences between the experiments were influenced not only by phosphorus form but also by meal composition: refined carbohydrates in Experiment 1 produced sharper early spikes, whereas the lower glycemic index of the dairy-based meal in Experiment 2 moderated initial rises and allowed phosphorus to exert a prolonged effect.

Glucose kinetics further demonstrated that phosphorus availability accelerated the return to baseline glucose levels. In both studies, phosphorus-supplemented participants returned to pre-prandial glucose concentrations more rapidly than placebo or chelated groups, underscoring the role of phosphorus in enhancing postprandial glucose homeostasis. The consistency between experiments also confirms that observed effects were specifically attributable to phosphorus rather than to other meal components (such as potassium or protein). This distinction is important given that Experiment 1 involved potassium phosphate, where potassium could have been a potential confounder, while Experiment 2 used dairy-derived phosphorus, in which other components such as whey protein might have influenced metabolism. The replication of effects across these distinct

experimental contexts strengthens the conclusion that phosphorus itself played a primary role in modulating postprandial glucose responses.

From an integrative perspective, these findings suggest a dual mechanism. Inorganic phosphorus appears to provide an immediate substrate for ATP production, rapidly blunting early glucose excursions, whereas organic phosphorus embedded within the dairy matrix sustains ATP availability, supporting prolonged glucose regulation. This highlights the importance of considering both bioavailability and the food matrix in dietary interventions aimed at optimizing postprandial glycemic control.

5.1.2 Observational evidence

The cross-sectional analysis of the UK NDNS dataset investigated whether habitual phosphorus intake was associated with glycemic regulation in free-living adults, specifically examining fasting glucose and glycated hemoglobin (HbA1c). In contrast to the acute postprandial effects observed in the clinical trials, no significant associations were found between habitual phosphorus intake and these steady-state glycemic markers. This indicates that, although phosphorus can modulate glucose dynamics in the short term, habitual intake at population levels may not produce detectable effects on fasting glucose or HbA1c.

These findings suggest that the acute improvements in postprandial glucose observed in controlled experiments do not necessarily translate into measurable differences in fasting glycemia in free-living populations, likely due to the influence of multiple dietary and lifestyle factors. Nonetheless, this does not diminish the relevance of phosphorus in glucose metabolism, as the acute postprandial effects observed experimentally may still contribute to long-term glycemic regulation when integrated over time.

5.1.3 Integration of evidence

Bridging experimental and observational data with insights from the systematic review provides a comprehensive perspective on the role of phosphorus in glycemic regulation. The systematic review highlighted that among studies assessing phosphorus supplementation, most reported minimal or no effects on glucose and insulin, often due to limitations such as infrequent blood sampling or small sample sizes. Only one study documented acute improvements in postprandial glucose and insulin, aligning with the acute effects observed in the present trials. Taken together, these lines of evidence suggest a temporal and context-dependent role of phosphorus in glycemic control. Experimental findings clearly demonstrate that phosphorus availability can modulate postprandial glucose excursions and accelerate glucose clearance, effects likely mediated via ATP-dependent pathways and enhanced substrate utilization. Observational evidence, while not capturing acute postprandial fluctuations, reinforces the importance of phosphorus in overall metabolic health and highlights that sustained intake, particularly from bioavailable sources like dairy, may support metabolic processes beyond glucose regulation.

Integrating these insights emphasizes the significance of phosphorus source, dose, and bioavailability. Organic phosphorus derived from dairy appears to confer more sustained postprandial benefits than isolated inorganic supplements, likely due to slower digestion, interactions with proteins such as casein, and a more gradual phosphate release. The systematic review supports this interpretation by showing that studies using poorly timed or isolated measurements frequently fail to detect these acute effects.

In summary, the experimental trials provide robust evidence for the acute modulatory role of dietary phosphorus on postprandial glucose, while observational findings suggest that habitual phosphorus intake may support broader metabolic health. The integration of these findings underscores the need for well-designed studies that can capture both acute and long-term metabolic outcomes, particularly with attention to the source, bioavailability, and timing of phosphorus consumption.

5.2 Dietary phosphorus and thermogenesis

5.2.1 Experimental evidence

Controlled clinical trials investigated the effect of dietary phosphorus on diet-induced thermogenesis (DIT) and energy expenditure. In Experiment 1, supplementation with 685 mg of inorganic phosphorus did not significantly alter core body temperature (CBT) or DIT compared to placebo, despite measurable changes in postprandial glucose. Similarly, Experiment 2, using dairy-derived organic phosphorus at a higher dose (970 mg per serving), did not produce detectable differences in postprandial thermogenesis, although it enhanced glucose regulation and modulated appetite-related measures.

These findings suggest that, under the experimental conditions tested, acute phosphorus intake may not directly affect systemic energy expenditure as measured by CBT. However, subtle effects on cellular metabolism, particularly ATP production in tissues such as liver, skeletal muscle, and adipose tissue, may not be fully captured by systemic thermogenic measures. Phosphorus-dependent ATP synthesis could theoretically influence mitochondrial efficiency, substrate cycling, and uncoupling

protein activity at the cellular level, all of which contribute to thermogenesis in a nuanced way.

5.2.2 Integration of evidence

Bringing together evidence from experimental trials and the systematic review, a coherent picture emerges. The systematic review included three trials administering 500 mg of inorganic phosphorus per serving (potassium phosphate) and reported measurable increases in energy expenditure and substrate oxidation. Importantly, these studies measured postprandial thermogenesis using indirect calorimetry, assessing oxygen consumption (VO_2) and carbon dioxide production (VCO_2), which are considered direct and sensitive markers of energy expenditure. In comparison, the clinical trials conducted as part of this research administered higher phosphorus doses—685 mg in Experiment 1 and 970 mg in Experiment 2—but measured thermogenesis via core body temperature using the CORE device, a less direct method. The discrepancy in measurement techniques may partly explain why significant thermogenic responses were observed in the systematic review studies but not in the current trials.

The systematic review suggested that phosphorus may influence energy metabolism through multiple pathways, including enhancing ATP synthesis, supporting oxidative phosphorylation, and modulating phosphocreatine turnover. While these mechanisms are physiologically plausible, their impact on whole-body thermogenesis appears modest or transient, consistent with the clinical trial data. The experimental evidence confirms that, although phosphorus significantly affects postprandial glucose regulation and appetite-related measures, its effect on CBT and DIT is limited under typical postprandial conditions. The absence of

observational data measuring energy expenditure underscores the need for mechanistic studies at the cellular and tissue level. In particular, skeletal muscle biopsies and hepatic ATP measurements could provide critical insights into phosphorus-mediated metabolic fluxes that are not captured by systemic thermogenesis measures.

5.3 Dietary phosphorus and appetite regulation

5.3.1 Experimental evidence

Both clinical trials demonstrated that dietary phosphorus can modulate appetite-related measures, even when systemic thermogenic responses were minimal. In Experiment 1, inorganic phosphorus supplementation was associated with reductions in subjective hunger ratings and delayed onset of postprandial hunger. Experiment 2, using dairy-derived organic phosphorus at a higher dose, showed similar effects on appetite suppression, with participants reporting greater satiety and lower desire to eat over the postprandial period.

These findings suggest that phosphorus may influence appetite through mechanisms partially independent of systemic energy expenditure. One plausible pathway is via ATP-mediated signaling in the liver and hypothalamus, where phosphorus availability supports hepatic ATP production that signals satiety. Phosphorus may also interact with key appetite-regulating hormones, including ghrelin, GLP-1, and PYY, enhancing postprandial satiety signals. Notably, these effects were observed even without detectable increases in core body temperature, indicating that appetite modulation is a sensitive and perhaps early marker of phosphorus's metabolic impact.

5.3.2 Integration of evidence

Considering evidence from the experimental trials alongside findings from the systematic review, a potential mechanistic link can be proposed. The systematic review indicated that phosphorus supplementation can influence appetite-regulating pathways via enhanced hepatic ATP production, increased energy availability in the hypothalamus, and modulation of satiety hormone responses. Experimental evidence supports this, as both inorganic and organic phosphorus consistently reduced subjective hunger and increased postprandial satiety, independent of detectable changes in systemic energy expenditure.

These findings highlight a critical distinction: phosphorus's effects on appetite may occur through fine-tuned cellular mechanisms that are not captured by conventional measures of energy expenditure such as CBT. Skeletal muscle and liver ATP turnover, hypothalamic energy sensing, and interactions with satiety hormones may all contribute to appetite regulation. Future research should focus on direct measurements of these pathways, including hepatic ATP concentrations, skeletal muscle phosphorus fluxes, and circulating satiety hormone profiles, to better understand the mechanistic underpinnings of phosphorus-induced appetite modulation.

5.4 Phosphorus in modern diet: real-world relevance

Phosphorus is an essential nutrient with critical roles in energy metabolism, cellular functions, and bone health. However, its metabolic effects and overall health outcomes depend not only on the total amount consumed but also on its dietary form, bioavailability, and the context of intake. Our research highlights two major considerations for understanding phosphorus

in modern diets and guiding future dietary recommendations: the source and form of phosphorus (organic versus inorganic) and the concept of phosphorus density in relation to energy intake.

Modern diets are increasingly dominated by highly bioavailable inorganic phosphorus, primarily found in processed foods, contrasting with the naturally occurring organic phosphorus in dairy, meats, and other whole foods. The bioavailability of these forms differs substantially: inorganic phosphorus is absorbed rapidly (≈90%), largely via passive paracellular transport, leading to swift increases in serum phosphate (Kalantar-Zadeh et al, 2010; Ratsma et al, 2024). While serum phosphate is tightly regulated under normal dietary conditions, excessive intake of inorganic phosphorus can disrupt mineral balance, including calcium homeostasis, potentially contributing to vascular calcification and elevated cardiovascular risk (Ritz et al, 2012; Peacock, 2021; Kemi et al, 2006; Jain & Elsayed, 2013; Chang et al, 2014).

In contrast, organic phosphorus from natural food sources is metabolized more slowly and efficiently, allowing for more stable postprandial phosphate kinetics. This distinction was reflected in our clinical trials: inorganic phosphorus supplementation (Experiment 1) reduced glucose levels only in the early postprandial phase, whereas dairy-derived organic phosphorus (Experiment 2) produced more sustained reductions across the entire postprandial period. These findings align with prior evidence suggesting that the source of phosphorus can differentially influence metabolic outcomes, including endothelial function, phosphate-regulating hormones such as FGF23, and postprandial glucose handling (Kawamura et al, 2018; Ratsma et al, 2024; McClure et al, 2020). Animal studies similarly demonstrate that inorganic phosphorus elevates serum phosphate and

increases risk markers for cardiovascular calcification, whereas organic phosphorus does not (Dobenecker et al, 2021; Coltherd et al, 2019).

Beyond source, the concept of phosphorus density—the amount of phosphorus per unit of energy consumed—is crucial. Phosphorus is central to ATP production and, therefore, to the efficient functioning of metabolic pathways involving glucose, carbohydrate, and lipid metabolism. Prioritizing phosphorus density ensures that phosphorus intake is aligned with energy needs, supporting optimal cellular energy metabolism rather than merely meeting total intake requirements. This approach is particularly relevant in modern diets, where caloric intake may fluctuate widely, and phosphate additives can contribute disproportionately to total phosphorus consumption.

Together, these considerations underscore the complexity of phosphorus's role in metabolism. Ensuring adequate intake from nutrient-dense, whole foods and maintaining an appropriate phosphorus-to-energy balance may enhance metabolic efficiency and support better long-term health outcomes. Importantly, although phosphorus is sometimes perceived as a nutrient with potential cardiovascular risks, current evidence is not conclusive (Winiarska et al, 2021). When sourced appropriately—favoring naturally occurring, bioavailable forms—phosphorus functions as an optimization mineral, supporting postprandial glucose regulation, diet-induced thermogenesis, and broader metabolic homeostasis, highlighting its potential to enhance rather than compromise metabolic health.

5.5 Future work

A. Cellular and tissue-level studies

The current findings highlight a critical need for research at the cellular and tissue levels to elucidate mechanisms by which phosphorus modulates postprandial metabolism. Skeletal muscle biopsies should be employed to assess mitochondrial function, ATP production, and substrate utilization in response to both acute and chronic phosphorus intake. Coupling these analyses with hepatic ATP measurements would allow quantification of phosphorus-mediated metabolic fluxes and provide insight into energy homeostasis, substrate cycling, and the role of phosphocreatine and other phosphorus-containing metabolites.

Integrative analyses combining these endpoints with metabolomics, protein phosphorylation, and metabolic flow assessments would allow mapping of phosphorus-dependent metabolic networks in detail. Such approaches could reveal subtle cellular effects not detectable through systemic measures, including regulatory pathways governing mitochondrial biogenesis, oxidative phosphorylation efficiency, and energy sensing via AMP-activated protein kinase (AMPK) pathways. Long-term interventions could further uncover chronic adaptations in tissue-specific ATP production, mitochondrial function, and metabolic flexibility.

B. Incorporation of endocrine and metabolic biomarkers

Future investigations should integrate endocrine and metabolic biomarkers, including insulin, GLP-1, PYY, and ghrelin, alongside markers of mitochondrial function and energy metabolism. This would provide a more comprehensive mechanistic understanding of phosphorus effects on postprandial metabolism and appetite regulation, addressing current limitations in linking systemic and cellular phosphorus effects and

elucidating hormonal and metabolic pathways underlying energy homeostasis, substrate utilization, and appetite control.

C. Comparative clinical trials of organic versus inorganic phosphorus

Future clinical studies should directly compare the metabolic effects of organic versus inorganic phosphorus under rigorously controlled conditions, matching macronutrient composition, phosphorus dose, participant characteristics, and study duration, while accounting for absorption and bioavailability differences. Trials should investigate the impact of phosphorus sourced from animal-based foods versus processed foods on postprandial glucose, insulin, and appetite-regulating hormones. Incorporating energy expenditure measurements via both systemic methods (e.g., indirect calorimetry) and cellular endpoints (e.g., skeletal muscle and liver analyses) will provide a more comprehensive understanding of phosphorus's role in metabolic regulation.

D. Measurement of serum phosphate and phosphorus density

Although serum phosphate levels are tightly regulated, their measurement in interventional studies comparing organic and inorganic phosphorus could provide valuable insight into phosphorus kinetics and tissue-specific utilization. Future studies should also explore a broader range of phosphorus density levels (mg per kcal) to better understand the relationship between dietary phosphorus relative to energy intake and metabolic outcomes. Developing validated dietary assessment tools to quantify inorganic phosphorus intake in free-living populations will be essential for linking experimental findings to habitual dietary patterns and informing public health guidelines.

E. Public health and observational research

Observational studies should prioritize source-specific phosphorus intake and phosphorus density rather than total intake alone, particularly focusing on highly bioavailable inorganic phosphorus from additives. Bridging experimental findings with population-level data will improve translation of mechanistic insights into dietary guidelines and public health recommendations. Development of robust tools for accurately quantifying inorganic phosphorus in dietary surveys will be key to this effort.

F. Exploratory and integrative approaches

Finally, exploratory interventions could push the boundaries of understanding phosphorus metabolism by integrating multiple layers of analysis across tissues, cells, and systemic physiology. Such studies could combine skeletal muscle and liver assessments with advanced measures of systemic energy expenditure, including indirect calorimetry and continuous glucose monitoring. Coupled with non-invasive imaging modalities, such as positron emission tomography (PET) or magnetic resonance spectroscopy (MRS), these studies could quantify tissue-specific substrate utilization, mitochondrial activity, and brown adipose tissue activation in response to dietary phosphorus. By leveraging these advanced methodologies, future research could delineate intricate interactions between dietary phosphorus and other nutrients, characterize its effects on appetite-regulating hormones, and define its influence on postprandial energy metabolism across both systemic and cellular scales, generating unprecedented mechanistic insight and informing targeted dietary strategies to optimize phosphorus intake. Ultimately, such approaches could enhance long-term metabolic health, refine evidence-based dietary guidelines, and

contribute to the prevention of metabolic disorders including type 2 diabetes, obesity, and metabolic syndrome.

5.6 Strengths and limitations

The principal strength of this project stems from its structured framework, which effectively combines theoretical, experimental, and observational approaches. This integrative design enables a more holistic understanding of the impact of phosphorus on postprandial metabolic responses, encompassing a systematic review, two complimentary clinical trials, and an analysis of the UK National Diet and Nutrition Survey.

Another key strength of this project lies in the integrative design of the two clinical trials, which were the first to investigate phosphorus metabolism under both conditions of deprivation and availability. By employing phosphorus chelation to restrict its presence and supplementation to enhance its intake, these studies provided a controlled framework to assess its metabolic effects, offering valuable insights into its role in glycemic regulation and energy metabolism.

The consistency of the results observed across replicate test days (Day 1 and Day 2) in both experiments represents another strength. Postprandial glycemia, CBT, and appetite-related measures exhibited similar trends across both test days, regardless of statistical significance. This consistency indicates that the observed outcomes were not influenced by day-to-day variations or experimental conditions but were instead driven by the effect of treatment. Such consistency strengthens the reliability and robustness of the findings, enhancing confidence in their reproducibility and ruling out potential confounding factors.

Having outlined the key strengths of this research, it is also important to acknowledge its limitations. The confines of each specific study are detailed in their respective chapters; however, several broader limitations warrant consideration in this section.

A key limitation of the clinical trials lies in the primary objective of the studies, which focused on investigating the relationship between phosphorus intake and postprandial metabolic responses, rather than directly comparing the effects of organic versus inorganic phosphorus. Consequently, the study design was not optimized to address this comparison, thereby limiting the robustness and precision of the conclusions regarding the differential effects of these phosphorus sources. However, the results obtained from these trials provide a foundation for future studies, enabling a more targeted and efficient comparison of the effects of organic versus inorganic phosphorus on metabolic outcomes.

Another notable limitation, both in the studies included in the systematic review and the clinical trials conducted, is the reliance on Visual Analog Scale questionnaires as the primary tool for appetite assessment. While widely validated in appetite research, these measures introduce a degree of subjectivity and potential variability. Furthermore, the absence of endocrine and metabolic measurements, namely GLP-1, PYY, insulin, and ghrelin, limits the ability to fully elucidate the mechanisms underlying phosphorus effects on postprandial metabolism and appetite regulation. An additional constraint is that serum phosphate levels were not measured in either experiment, limiting the ability to directly assess phosphorus homeostasis and compare the effects of bioavailability and source classification between the two experimental conditions.

In the NDNS analysis, phosphorus intake was assessed without differentiating between organic and inorganic sources. This limitation hinders the ability to evaluate the distinct effects of inorganic phosphorus, primarily derived from food additives and processed foods, compared to naturally occurring dietary phosphorus. A more precise classification of phosphorus sources would have provided deeper insights into their respective associations with the individual components of metabolic syndrome.

5.8 Conclusion

Collectively, the findings from this dissertation provide novel insights into the role of phosphorus in postprandial metabolism. The systematic review synthesized existing evidence on phosphorus and its influence on glycemia, energy metabolism, and other metabolic outcomes, highlighting both the potential and limitations of dietary phosphorus in metabolic regulation.

The controlled clinical trials demonstrated that both inorganic and organic dietary phosphorus significantly modulated postprandial glucose response, with a more pronounced and sustained effect observed with organic phosphorus derived from dairy sources. While dietary phosphorus intake did not significantly impact core body temperature, it was found to reduce appetite-related measures, indicating a potential role in diet-induced thermogenesis and energy balance. These findings contribute to the growing body of literature on the relationship between phosphorus and energy metabolism, emphasizing its relevance in maintaining metabolic homeostasis.

The cross-sectional analysis of the UK NDNS dataset revealed that higher habitual phosphorus intake is associated with improved metabolic markers, particularly lower diastolic blood pressure and triglyceride levels, and a reduced risk of metabolic syndrome, further supporting the importance of phosphorus in metabolic health.

A key takeaway of this research is the importance of considering phosphorus intake relative to energy consumption, highlighting the concept of phosphorus density as a more meaningful metric than total intake alone. Additional insights include the differential effects of organic versus inorganic phosphorus, the potential benefits of prioritizing naturally occurring phosphorus sources, and the interplay between phosphorus intake, postprandial glucose regulation, and appetite modulation. Collectively, these findings underscore the need for dietary guidelines that account for both the source and density of phosphorus to optimize metabolic outcomes.

Overall, this dissertation provides compelling evidence for the role of phosphorus in human metabolism and offers a foundation for future research to further elucidate its mechanistic effects, refine dietary recommendations, and explore its potential therapeutic applications in metabolic diseases such as type 2 diabetes, obesity, and metabolic syndrome.

SUPPLEMENTARY MATERIALS

Supplementary table 1. Databases' search strategy used for the systematic review.

Database	Search strategy	Citations
Cochrane Central Register	***ID Search Hits*** 1. Dietary phosphorus in Trials (N = 403) 2. MeSH descriptor: [Phosphorus] explode all trees (N = 442) 3. (Phosphorus): TI (title field), AB (abstract field) , KW (keyword field) in Cochrane Reviews, Trials (Word variations have been searched) (N = 1071)	3582
PubMed	((("phosphorus, dietary"[All Fields] OR "phosphorus, dietary"[MeSH Terms] OR dietary phosphorus[Text Word] OR "phosphorus"[All Fields] OR "phosphorus"[MeSH Terms] OR phosphorus[Text Word]) AND ("Clinical Trial" [Publication Type] OR "Clinical Trials as Topic"[Mesh] OR "Controlled Clinical Trial" [Publication Type] OR "Clinical Trial Protocols as Topic"[Mesh] OR "Non-Randomized Controlled Trials as Topic"[Mesh] OR "Pragmatic Clinical Trial" [Publication Type] OR "Clinical Trial, Phase IV" [Publication Type] OR "Adaptive Clinical Trial" [Publication Type] OR "Adaptive Clinical Trials as Topic"[Mesh] OR "Pragmatic Clinical Trials as Topic"[Mesh] OR "Evaluation Study" [Publication Type]))	3461
Google Scholar	(dietary phosphorus | phosphorus) AND (clinical trial | controlled clinical trial | placebo | controlled | trial | non-randomized | randomized | crossover)	980

Supplementary table 2. Pairwise comparisons of mean phosphorus intake (mg) from main food groups.

Sex	Food group 1	Food group 2	Mean 1	Mean 2	Adjusted p-value[a]
Males	Beverages	Bread, grains, and cereal products	110.12	262.42	<0.0001
	Beverages	Meat, poultry, fish, and eggs	110.12	379.92	<0.0001
	Beverages	Milk and dairy	110.12	292.00	<0.0001
	Beverages	Vegetables	110.12	82.37	<0.0001
	Bread, grains, and cereal products	Meat, poultry, fish, and eggs	262.42	379.92	<0.0001
	Bread, grains, and cereal products	Milk and dairy	262.42	292.00	<0.0001
	Bread, grains, and cereal products	Vegetables	262.42	82.37	<0.0001
	Meat, poultry, fish, and eggs	Milk and dairy	379.92	292.00	<0.0001
	Meat, poultry, fish, and eggs	Vegetables	379.92	82.37	<0.0001
	Milk and dairy	Vegetables	292.00	82.37	<0.0001
Females	Beverages	Bread, grains, and cereal products	56.28	206.95	<0.0001
	Beverages	Meat, poultry, fish, and eggs	56.28	275.10	<0.0001
	Beverages	Milk and dairy	56.28	274.17	<0.0001
	Beverages	Vegetables	56.28	79.34	<0.0001
	Bread, grains, and cereal products	Meat, poultry, fish, and eggs	206.95	275.10	<0.0001
	Bread, grains, and cereal products	Milk and dairy	206.95	274.17	<0.0001
	Bread, grains, and cereal products	Vegetables	206.95	79.34	<0.0001
	Meat, poultry, fish, and eggs	Milk and dairy	275.10	274.17	0.17
	Meat, poultry, fish, and eggs	Vegetables	275.10	79.34	<0.0001
	Milk and dairy	Vegetables	274.17	79.34	<0.0001

[a] P-values less than 0.05 are considered statistically significant.

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APPENDIX A

TITLE ABSTRACT SCREENING FORM

1. Does the study assess the effect of dietary phosphorus intake? If yes, include.
2. Is the population adults 18 – 64 years of age? If yes, include.
3. Is the population health? If yes, include.
4. Is the study a systematic review? If yes, exclude.

Author	Year	Title	Study design	Sample size	Intervention	Dosage	Duration	Outcome

APPENDIX B

DATA EXTRACTION FORM

Author	Study design	Study aim	Population	Duration	Intervention	Measured Outcomes	Findings

APPENDIX C

EXPERIMENT 1 CONSENT FORM

Consent to participate in a research study

Title of Research Study: The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet Induced Thermogenesis

Experiment 1: *Assessing the magnitude of carbohydrate metabolism on energy expenditure, in the presence of phosphorus.*

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Co-Investigators: Rania El Khoury, Karen Zoghbi

Address: American University Beirut, Hamra, Beirut – Lebanon/01 – 350 000

Site where the study will be conducted: American University of Beirut-Department of Nutrition and Food Science.

You are likely aware of this study through your contact with our co-investigators who briefly described the study design upon receiving an invitation email script and/or through your individual interest for participation in this **research study** after seeing our hung posters on your faculty's billboard. Before agreeing to participate in this study, it is important that you read the information below. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. Also described are the alternative procedures, if any, available to you, as well as your right to withdraw from the study at any time. You should feel free to ask any questions that you may have.

Purpose of the overall research: Phosphorus is a mineral that is naturally present in our foods and is required by our bodies for normal function. It is known to have an essential role in an individual's overall metabolism, whereby a compromise in its status may indirectly contribute to the onset of several metabolic conditions, such as insulin resistance and obesity. The overall objective of this study is to measure the impact of dietary phosphorus on carbohydrates and energy metabolism via the continuous monitoring of blood glucose levels and body temperature. To entirely serve this objective, macronutrient composition and phosphorus content of standardized meals will be manipulated in the course of three sequential experiments. Changes in post prandial glucose levels, body temperature, and heat flux will subsequently be measured.

This research project will include **three** different interventional experiments. You may choose to participate in one or more of the three experiments.

A. **Purpose of Experiment 1**: to determine the magnitude of carbohydrate metabolism on energy expenditure, in the presence of phosphorus. **Project/Procedures Description:** A researcher will contact you to go over the study procedures and go through a pre-screening evaluation questionnaire with you to check if it is safe for you to participate. The estimated time for the pre-screening questionnaire administration will be around 20 to 30 minutes. If you meet the preliminary inclusion criteria, you will be asked to show up for a screening visit within the nearest time. During this visit, we will take your anthropometric measurements (weight, height, and BMI), body composition analysis test (a detailed breakdown of your weight in terms of muscle, fat, and water)a finger-prick blood test to rule out diabetes or prediabetes as well as a urine test to rule out kidney failure. Body composition will be assessed using the *Inbody 770* machine, whereby you will have to stand still and align your feet with the foot electrolytes while grabbing the handles. Your body measurements will then be automatically generated. Urine samples will be analyzed at the American University of Beirut. The estimated time for this screening visit will be also about 20 to 30 minutes. We will walk you through the study's timeline and protocol. If you are still happy to take part, you will then be asked to sign this consent form. This experiment requires a sample size of 16 (8 males and 8 females).Exclusion criteria for this experiment include: overweight or obesity, any significant medical diseases including renal failure diabetes and pre-diabetes, lactose intolerance or allergies to milk, eggs and/or dairy foods, having done any bariatric surgeries, history of kidney stones, history or current psychiatric illness, weight loss of 3% or more in the preceding 3 months, pregnancy or lactation.

The duration of this experiment is 10 days. For this experiment, you will be asked to attend multiple visits at the Faculty of Agriculture and Food Sciences/ American University of Beirut, as illustrated in the Figure below. For each of the visits (except for the end visit), you will be asked to come in the morning following a 12-hour overnight fast, and consume a breakfast meal at the facility. You will have to refrain from engaging in any strenuous physical activity and alcohol consumption, one day prior to each visit.

On your first visit, a trained study staff will insert a glucose monitoring device and a body temperature device. The temperature monitoring device is totally non-invasive. It can be comfortably worn and attached to a strap around the chest area. It continuously measures real-time core body temperature.

The glucose monitoring device is a water-resistant device that gets inserted beneath the abdomen's skin. The insertion process is simple and pain-free. It provides continuous glucose readings that get updated every 5 minutes.

Study staff will instruct you in a one-to-one session on the use of these wearable devices, their respective mobile applications, and overall compliance, noting that the glucose monitoring device is inserted by trained staff and under normal circumstances, you should not remove it during the 10-day study period. Please note that you will have to download the core mobile application to allow for measurements of core body temperature. As for the glucose monitoring device (Dexcom), you will be supplied with receivers to detect glucose readings since their mobile application is not compatible with all phone types. As such, you will also have to always keep the receiver with you. The body temperature device is

easy to displace but you are advised to keep it in the required position for optimized temperature recording. In case any abnormal values appear, the research team will immediately detect them and will message you to make sure that the devices worn are well positioned and that your phone was within 6 meters with no obstacles (like walls or metal). Noting that this distance should be further reduced if there is water between the glucose monitoring device and receivers (for example, while showering or swimming). If this is not the case, you will be advised to consult your family doctor. In case of any adverse events arise as a result of the current protocol, HIP should be covering for any "medical treatment" that might be needed. You will also be asked and trained to log your daily dietary intake over the 10-day study period, on the food data record sheets which will be provided to you. On these sheets, you will have to record your daily dietary intake along with the exact time of ingestion, ingredient quantities, and physical activity records. You will then have to return these filled data sheets to the co-investigators on the 4^{th} and 9^{th} day of the study. You will surely have the chance to ask any questions and clarify any confusions in case of any. This visit should take approximately 60 minutes in total.

On your subsequent visits, you will consume your offered breakfast meals (600 kcal) while wearing the continuous glucose and temperature monitoring devices. These visits should take approximately 30 minutes. You will be asked to stay stationary and fast for 3 hours after the ingestion of every breakfast meal, after which you may eat as much you want, while recording your entire daily intake. To help us know better about your appetite during this experiment, you will be provided with appetite score sheets to fill in every 15 minutes for 2 hours after ingestion of the breakfast meal.

On your last/end visit, you will just be visiting the facility for the removal of your glucose and body temperature monitoring devices.

The breakfast meals offered on your visits will be safely prepared and packaged in the Pilot Plant [Department of Nutrition and Food Sciences/ American University of Beirut]. For this experiment, your breakfast meal will be composed of a refined carbohydrate meal shake, which will consist of granulated sugar, corn oil, dried egg white powder, water and a few drops of artificial flavor, ingested with one of the tablets below:

- Potassium phosphate tablet: which will be used as the source of oral phosphorus supplementation. Dosage used is 600 mg (1mg/1kcal/meal)
- Empty phosphorus-free placebo pill.

B. **Duration:** The estimated time to complete this experiment is 10 days. You may leave the study at any time. If you decide to stop participating, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with AUB.

C. **Risks and discomforts:** We do not expect that there will be any risks associated with taking part in this study, except for the time commitment and compliance required of you. There will be some minor discomfort related to the wearable glucose and temperature monitoring devices. The temperature monitoring device is totally non-invasive. The glucose monitoring device however, monitors activity via the use of a slim

sensor, which gets inserted beneath the abdomen's skin, by a one-touch auto-applicator. The sensor insertion process is simple and pain-free and will only be inserted by fully trained research staff, in order to reduce any potential risks of discomfort. The potential side effects of high phosphorus intake are nausea, diarrhea, or epigastric pain; from our experience the use of this dose was not associated with any of these signs. If you predict that you may be pregnant or if you became pregnant during the study, it may involve currently unforeseeable risks to your embryo.

D. **Benefits:** Although there are no immediate benefits for you from being part in this research project, it is hoped that this work will have a beneficial impact on the advances of human nutrition in improving metabolism and glucose tolerance; ultimately preventing the development of insulin resistance and obesity, which have been classified as global chronic threats.

E. **Confidentiality:** To secure the confidentiality of your responses, your name and other identifiers will never be attached to your answers. All codes and data will be kept in a locked drawer in a locker room or in a password protected computer that is kept secure. Data access is limited to the Principal investigator and researchers working directly on the project. All data will be destroyed responsibly after the required retention period, which is a minimum period of three years - in line with the University's expectations. In case of withdrawal from the study, all data collected from you up to the point of withdrawal will remain part of the study database and may not be removed. Your privacy will be maintained in all published and written data resulting from this study. Your name or other identifying information will not be used in our reports or published

papers. If you decide to withdraw from the study, our investigators will ask you if you wish to provide further data collection from routine medical care. Designated individuals of the American University of Beirut and the University of Nottingham may be given access to data for monitoring and/or audit of the study to ensure we are complying with guidelines. This research study will be written up as a dissertation for the degree of PhD in Clinical Nutrition and will be registered on ClinicalTrials.gov. Upon successful submission of the dissertation, we ultimately aim to openly share the knowledge resulting from this research project with the public, and hence the research may also be published in scientific journals. Publications will not contain any identifiable personal data. Your privacy will be protected and maintained in all written and published data.

F. Compensation/Incentive: You will be given Amazon vouchers of $40 as reimbursements for expenses incurred as a result of your participation in each of the three experiments ($40 per experiment). These shall include transportation costs, parking fees, and lost work time fees. The study will be done at absolutely no cost from you.

G. Payment for Research-related Injury: This study is associated with minimal risk. However, there may be unforeseeable risks. In case of any adverse event as a result of the study, you will be covered by the Health Insurance Plan (HIP), provided to you by the AUB. In the unlikely event of any adverse event as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by the HIP.

If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

H. Contact Information and Questions:

1) If you have any questions or concerns about the research, do not hesitate to contact the principal investigator:

Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb

OR Rania El Khoury, 03999896, ree10@aub.edu.lb

2) If you have any questions, concerns or complaints about your rights as a participant in this research, feel free to contact the following office at AUB: Biomedical Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5445

I. Participant Rights: Participation in this study is voluntary. You are free to leave the study at any time without any negative consequences and penalty. Your decision not to participate does not influence your relationship with AUB. Please note that the investigator has the right to terminate your participation in the study, under circumstances of unethical conduct and/or disrespectful behavior. In case of such misconduct, you will be informed over the phone or face to face about the termination of your participation. In case of any research risks occurring, your participation will also be terminated and any adverse effect you may face as a result will be reported to IRB and covered by HIP. Your compensation will then be determined depending on how far you have reached in the study.

Do you wish to participate in this study? Yes _____ No _____

J. Future Contact

Would you like to be contacted for future research?

Yes _____ No _____

H- Patient's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Omar Obeid at Tel: +961-1-350 000 Ext: 4440 or any of his/her designee involved in the study in case of any questions. If I feel that my questions have not been answered, I can contact the Institutional Review Board for human rights at +961-1350000 Ext: 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care or benefits. I know that I will receive a copy of this signed informed consent.

_____	_____	_____
Name of Patient	Signature	Date & Time
_____	_____	_____
Witness's Name	Signature	Date & Time

K- Documentation of Consent:

_____	_____	_____
Name of Person obtaining Consent	Signature	Date & Time

APPENDIX D

EXPERIMENT 2 CONSENT FORM

Consent to participate in a research study

Title of Research Study: The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet Induced Thermogenesis

<u>Experiment 1</u>: ***Assessing the independent effect of dietary phosphorus on protein metabolism and energy expenditure, in the presence of phosphorus***

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Co-Investigators: Rania El Khoury, Karen Zoghbi

Address: American University Beirut, Hamra, Beirut – Lebanon/01 – 350 000

Site where the study will be conducted: American University of Beirut-Department of Nutrition and Food Science.

You are likely aware of this study through your contact with our co-investigators who briefly described the study design upon receiving an invitation email script and/or through your individual interest for participation in this **research study** after seeing our hung posters on your faculty's billboard. Before agreeing to participate in this study, it is important that you read the information below. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. Also described are the alternative procedures, if any, available to you, as well as your right to withdraw from the study at any time. You should feel free to ask any questions that you may have.

Purpose of the overall research: Phosphorus is a mineral that is naturally present in our foods and is required by our bodies for normal function. It is known to have an essential role in an individual's overall metabolism, whereby a compromise in its status may indirectly contribute to the onset of several metabolic conditions, such as insulin resistance and obesity. The overall objective of this study is to measure the impact of dietary phosphorus on carbohydrate and energy metabolism via the continuous monitoring of blood glucose levels and body temperature. To entirely serve this objective, macronutrient composition and phosphorus content of standardized meals will be manipulated in the course of three sequential experiments. Changes in post prandial glucose levels, body temperature, and heat flux will subsequently be measured. This research project will include **three** different interventional experiments. You may choose to participate in one or more of the three experiments.

Purpose of Experiment 2: to measure the effect of phosphorus on protein metabolism and consequently on energy expenditure, in addition to investigating the independent impact of phosphorus found in dairy foods on glycaemia of healthy individuals.

A. Project/Procedures Description: A researcher will contact you to go over the study procedures and go through a pre-screening evaluation questionnaire with you to check if it is safe for you to participate. The estimated time for the pre-screening questionnaire administration will be around 20 to 30 minutes. If you meet the preliminary inclusion criteria, you will be asked to show up for a screening visit within the nearest time. During this visit, we will take your anthropometric measurements (weight, height, and BMI), body composition analysis test (a detailed breakdown of your weight in terms of muscle, fat, and water), a finger-prick blood test to rule out diabetes or prediabetes as well as a urine test to rule out kidney failure. Body composition will be assessed using the *Inbody 770* machine, whereby you will have to stand still and align your feet with the foot electrolytes while grabbing the handles. Your body measurements will then be automatically generated. Urine samples will be analyzed at the American University of Beirut. The estimated time for this screening visit will be also about 20 to 30 minutes. We will walk you through the study's timeline and protocol. If you are still happy to take part, you will then be asked to sign this consent form.

This experiment requires a sample size of 16 (8 males and 8 females). Exclusion criteria for this experiment include: overweight or obesity, any significant medical disease, including renal failure diabetes and pre-diabetes, lactose intolerance or allergies to milk, eggs and/or dairy foods, having done any bariatric surgeries, history of kidney stones, history or

current psychiatric illness, weight loss of 3% or more in the preceding 3 months, pregnancy or lactation.

The duration of this experiment is 10 days. For this experiment, you will be asked to attend multiple visits at the Faculty of Agriculture and Food Sciences/ American University of Beirut, as illustrated in the Figure below. For each of the visits (except for the end visit), you will be asked to come in the morning following a 12-hour overnight fast, and consume a breakfast meal at the facility. You will have to refrain from engaging in any strenuous physical activity and alcohol consumption, one day prior to each visit.

On your first visit, a trained study staff will insert a glucose monitoring device and a body temperature device. The temperature monitoring device is totally non-invasive. It can be comfortably worn and attached to a strap around the chest area. It continuously measures real-time core body temperature.

The glucose monitoring device is a water-resistant device that gets inserted beneath the abdomen's skin. The insertion process is simple and pain-free. It provides continuous glucose readings that get updated every 5 minutes.

Study staff will instruct you in a one-to-one session on the use of these wearable devices, their respective mobile applications, and overall compliance, noting that the glucose monitoring device is inserted by trained staff and under normal circumstances, you should not remove it during the 10-day study period. Please note that you will have to download the core mobile application to allow for measurements of core body temperature. As for the glucose monitoring device (Dexcom), you will be

supplied with receivers to detect glucose readings since their mobile application is not compatible with all phone types. As such, you will also have to always keep the receiver with you. The body temperature device is easy to displace but you are advised to keep it in the required position for optimized temperature recording. In case any abnormal values appear, the research team will immediately detect them and will message you to make sure that the devices worn are well positioned and that your phone was within 6 meters with no obstacles (like walls or metal). Noting that this distance should be further reduced if there is water between the glucose monitoring device and receivers (for example, while showering or swimming). If this is not the case, you will be advised to consult your family doctor. In case of any adverse events arise as a result of the current protocol, HIP should be covering for any "medical treatment" that might be needed.

You will also be asked and trained to log your daily dietary intake over the 10-day study period, on the food data record sheets which will be provided to you. On these sheets, you will have to record your daily dietary intake along with the exact time of ingestion, ingredient quantities, and physical activity records. You will then have to return these filled data sheets to the co-investigators on the 4^{th} and 9^{th} day of the study. You will surely have the chance to ask any questions and clarify any confusions in case of any. This visit should take approximately 60 minutes in total.

On your subsequent visits, you will consume your offered breakfast meals (600kcal) while wearing the continuous glucose and temperature monitoring devices. These visits should take approximately 30 minutes. You will be asked to stay stationary and fast for 3 hours after the ingestion of every breakfast meal, after which you may eat as much you want, while

recording your entire daily intake. To help us know better about your appetite during this experiment, you will be provided with appetite score sheets to fill in every 15 minutes for 2 hours after ingestion of the breakfast meal. On your last/end visit, you will just be visiting the facility for the removal of your glucose and body temperature monitoring devices.

The breakfast meals offered on your visits will be safely prepared and packaged in the Pilot Plant [Department of Nutrition and Food Sciences/ American University of Beirut]. For this experiment, your breakfast meal will be composed of a dairy meal shake which will consist of full fat powdered milk, granulated sugar, corn oil, dried egg white powder, water and a few drops of artificial flavor, ingested with one of the tablets below:

- Phosphate binding tablet: Sevelamer Carbonate or Renvela, which will be used to prevent the absorption of phosphorus from your meal. Dosage used is 800 mg/meal.
- Empty phosphorus-free placebo pill

A. **Duration:** The estimated time to complete this experiment is 10 days. You may leave the study at any time. If you decide to stop participating, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with AUB.

B. **Risks and discomforts:** We do not expect that there will be any risks associated with taking part in this study, except for the time commitment and compliance required of you. There will be some minor discomfort related to the wearable glucose and temperature monitoring devices. The

temperature monitoring device is totally non-invasive. The glucose monitoring device, however, monitors activity via the use of a slim sensor, which gets inserted beneath the abdomen's skin, by a one-touch auto-applicator. The sensor insertion process is simple and pain-free and will only be inserted by fully trained research staff, in order to reduce any potential risks of discomfort. The phosphate binding tablets are orally administered pharmaceuticals, widely approved for the treatment of hyperphosphatemia. They are efficacious in preventing phosphate absorption from meals, without causing any metabolic or gastrointestinal side effects or discomfort in the short term. In fact, most of the safety experience is with sevelamer carbonate tablets, as mentioned in its prescribing information. Some of the adverse effects usually associated with its long-term use in studies include vomiting (22%), nausea (20%), diarrhea (19%), dyspepsia (16%), abdominal pain (9%), flatulence (8%), and constipation (8%). If you predict that you may be pregnant or if you became pregnant during the study, it may involve currently unforeseeable risks to your embryo.

C. **Benefits:** Although there are no immediate benefits for you from being part in this research project, it is hoped that this work will have a beneficial impact on the advances of human nutrition in improving metabolism and glucose tolerance; ultimately preventing the development of insulin resistance and obesity, which have been classified as global chronic threats.

D. **Confidentiality:** To secure the confidentiality of your responses, your name and other identifiers will never be attached to your answers. All codes and data will be kept in a locked drawer in a locker room or in a password protected computer that is kept secure. Data access is limited to the Principal

investigator and researchers working directly on the project. All data will be destroyed responsibly after the required retention period, which is a minimum period of three years - in line with the University's expectations. In case of withdrawal from the study, all data collected from you up to the point of withdrawal will remain part of the study database and may not be removed. Your privacy will be maintained in all published and written data resulting from this study. Your name or other identifying information will not be used in our reports or published papers. If you decide to withdraw from the study, our investigators will ask you if you wish to provide further data collection from routine medical care. Designated individuals of the American University of Beirut and the University of Nottingham may be given access to data for monitoring and/or audit of the study to ensure we are complying with guidelines. This research study will be written up as a dissertation for the degree of PhD in Clinical Nutrition, and will be registered on ClinicalTrials.gov. Upon successful submission of the dissertation, we ultimately aim to openly share the knowledge resulting from this research project with the public, and hence the research may also be published in scientific journals. Publications will not contain any identifiable personal data. Your privacy will be protected and maintained in all written and published data.

E. **Compensation/Incentive:** You will be given Amazon vouchers of $40 as reimbursements for expenses incurred as a result of your participation in each of the three experiments ($40 per experiment). These shall include transportation costs, parking fees, and lost work time fees. The study will be done at absolutely no cost from you.

F. **Payment for Research-related Injury:** This study is associated with minimal risk. However, there may be unforeseeable risks. In case of any adverse event as a result of the study, you will be covered by the Health Insurance Plan (HIP), provided to you by the AUB. In the unlikely event of any adverse event as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by the HIP.

If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

G. **Contact Information and Questions:**

1) If you have any questions or concerns about the research, do not hesitate to contact the principal investigator:

Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb

OR Rania El Khoury, 03999896, ree10@aub.edu.lb

2) If you have any questions, concerns or complaints about your rights as a participant in this research, feel free to contact the following office at AUB: Biomedical Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5445

H. Participant Rights: Participation in this study is voluntary. You are free to leave the study at any time without any negative consequences and penalty. Your decision not to participate does not influence your relationship with AUB. Please note that the investigator has the right to terminate your participation in the study, under circumstances of unethical conduct and/or disrespectful behavior. In case of such misconduct, you will be informed over the phone or face to face about the termination of your participation. In case of any research risks occurring, your participation will also be terminated and any adverse effect you may face as a result will be reported to IRB and covered by HIP. Your compensation will then be determined depending on how far you have reached in the study.

Do you wish to participate in this study?

Yes _____ No _____

I. Future Contact

Would you like to be contacted for future research?

Yes _____ No _____

J. Patient's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Omar Obeid at Tel: +961-1-350 000 Ext: 4440 or any of his/her designee involved in the study in case of any questions. If I feel that my questions have not been answered, I can contact the Institutional Review Board for human rights at +961-1350000 Ext: 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care or benefits. I know that I will receive a copy of this signed informed consent.

_____	_____	_____
Name of Patient	Signature	Date & Time
_____	_____	_____
Witness's Name	Signature	Date &Time

K- Documentation of Consent:

_____	_____	_____
Name of Person obtaining Consent	Signature	Date & Time

APPENDIX E

ADVIRTISEMENT POSTER

The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet Induced Thermogenesis

Do you want to participate in a research study conducted at American University of Beirut?

Participation Criteria

If your age is between 18 and 64 years

If you were in good health and do not suffer from diabetes or kidney disease

JOIN US!!!

Purpose:

The purpose of this study is to measure the impact of dietary phosphorus on carbohydrate and energy metabolism via the continuous monitoring of blood glucose levels and body temperature.

You are expected to show up 6 times at FAFS department at 8:00 am (after an overnight fast) to have a breakfast meal, and stay for approximately an hour. You will be wearing a device that monitors your blood glucose for ten consecutive days, and another device that monitors your core body temperature for six days.

Principal investigator: *Dr. Omar Obeid*

For participation, please feel free to contact any of the following investigators:

Mrs. Rania El Khoury - 03 999 896 – ree10@aub.edu.lb

Miss Karen Zoghbi - 70 112 818 – kfz03@mail.aub.edu

APPENDIX F

PRE-SCREENING DATA COLLECTION SHEET

Pre-Screening Data Collection Form

Project Title: The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet Induced Thermogenesis.

Principal investigator: Dr Omar Obeid - Tel: 00961-1-350 000 Ext 4440; email:oo01@aub.edu.lb)

The estimated time to complete this questionnaire is approximately 20 minutes.

1. Name:_____
2. Subject ID: _____
3. Date of birth:_____;
4. (Age: _____ years old)
5. Sex: ☐ Male ☐ Female
6. For females only:

   Are you currently pregnant or could possibly be pregnant?

   Are you currently lactating?

   _____
7. Do you have HIP coverage? ☐ Yes ☐ No
8. Weight: _____ kg

   BMI: _____kg/m^2
9. Height: _____ cm
10. Have you lost weight recently? ☐ Yes ☐ No

- If yes what was your weight 3 months ago?

  _____kg % weight loss: _____

11. Have you ever done any surgery?

    _____

12. Do you suffer from any health problem or disease?

    _____

13. Have you ever experienced kidney stones?

    _____

14. Do you currently suffer or have ever suffered of any psychiatric illness?

    _____

15. Do you take any medication or supplement?

    _____

16. Do you have any food allergy? (Especially Allergies to milk, eggs and/or dairy foods)

    _____

17. Do you have any food intolerance?

    _____

18. Are you willing to maintain your regular dietary and physical activity habits throughout study period?

    _____

19. Do you have a smartphone?

    ☐ Yes ☐ No

20. Are you willing to learn how to use 2 mobile applications?

    ☐ Yes ☐ No

21. Are you traveling anytime during the 10 days experimental time period?

    ☐ Yes ☐ No

APPENDIX G

FOLLOW UP MESSAGE

Documentation of Participants' Follow up Message

Dear Participant,

As you are taking part in the study entitled 'The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet-Induced Thermogenesis', we have noticed some recent abnormal values in your data. Hence, we are currently messaging you in order to make sure that the devices worn are well positioned and that your phone remains within a 6 meters distance - with no obstacles (like walls or metal). Kindly keep note that this distance should be further reduced if there is water between your Dexcom device and the receiver (for example, while showering or swimming).
Please let us know if any of the above happened.

If the answer is that the device was misplaced or that the participant was using water or far from their phone, the next message will be:
Please make sure that the device stays in the advised position.
or
Please make sure to put your phone and/or receiver as close as possible to you while having water close to the device.
or
Please make sure to stay within 6 meters of your phone and/or receiver or less in case of showering and/or swimming.

If the answer was that none of the above happened, the next message will be:
We have noticed abnormal (specify if low or high) sugar levels in your record; we advise you to consult with your family doctor in the nearest time.

Thank you,

Warmest regards from the research team

APPENDIX H

DEVICES PROTOCOL EXPLANATION

Draft of the key points related to wearable devices discussed with participants during the Baseline Visit

Core Device

- This is the core body device that is designed to measure the core temperature of your body and your skin temperature.
- Non-invasive device so there will be no needles, no prick. Not feel any pain. Will be simply in contact with your skin and measure temperature.
- To be able to use the device, we need to download an application on your phone and create an account.
- The account is protected by password, and we need to have the password of your account so we can always monitor.
- We will have access to all the data that is recorded by the device. The only thing we can see even if you are sharing the device with us is the temperature of your body. We will not have access to any private information that you have stored on your phone.
- The device operates using Bluetooth so the Bluetooth on your phone should be turned on all the time. You cannot turn it off.
- For us to receive the data we will need the WIFI signal so make sure every now and then you are connected to the Wi-Fi so that we can receive updates.
- The phone should be always kept close to you even when you are in the shower.
- The device you should be always wearing even when you are showering or when swimming or sleeping. Avoid swimming prolonged period.
- Show them how the device will work. After 3 days of wearing the device, you need to charge it. Remove it from the body, recharge it and wear it after the washout period is done.

  on the body, you will feel a small prick, but you shouldn't be in any pain or discomfort. So, if you feel pain at any point, you should let us know.
- The Dexcom will send the measurements to a small receiver. You must always carry them and need to have a Dexcom account so you can share the data with us. Just like the core, the only data we can see is the glucose values in the bodies.
- You are not allowed to touch anything on the receiver. The only thing they can check is data alarm and the screen in that case also gives instructions how to shut of the alarm press 1 and then 2. Other than that, do not change setting. Don't change notifications.
- This device you must wear it for 10 days. You can shower with it. They cannot swim for prolonged period. Put the receiver in a Ziploc bag so that it's not affected by moisture and the put it near them. Not in the bath. Near the mirror or chair.
- There shouldn't be any walls between them and the device. You should check the battery of the device during the 10 days. Once the battery is low, you must recharge it.
- The device for the first 8 hours will keep giving low readings because still calibrating. Lot of alarms. It doesn't mean your glucose is low. It means it still adjusting.
- You need to always make sure the devices are handled well and protected because they are very expensive and will be used by more than 1 participant and cannot afford to be malfunctioned.

They should always keep communicating with us if they have questions and if they feel anything. You have our phone numbers and can tell us immediately.

APPENDIX I

FOOD RECORD SHEET

Date: ___/___/2023

Project Title: The Impact of Dietary Phosphorus on Postprandial Carbohydrate Metabolism and Diet Induced Thermogenesis.

Principal investigator: Dr Omar Obeid - Tel: 00961-1-350 000 Ext 4440; email: oo01@aub.edu.lb)

FOOD RECORD:

Time of meal/snack	Type of food	Amount eaten	Comments (if any)

PHYSICAL ACTIVITY:

Time of work-out	Type work-out	Duration of work-out	Comments (if any)

APPENDIX J

VISUAL ANALOG SCALE QUESTIONNAIRE

(Organoleptic properties)

Preload: **Time:**

Questions on palatability of the test meal:

Visual appeal

Good _____ Bad

Smell

Good _____ Bad

Taste

Good _____ Bad

Aftertaste

Good _____ Bad

Palatability

Good _____ Bad

APPENDIX K

VISUAL ANALOG SCALE QUESTIONNAIRE

(Appetite-related measures)