Degree project

UNIVERSITY OF SKÖVDE

Investigation Of Physiological And Gene Expression Responses To A Low Carbohydrate High Protein Dietary Intervention

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Abstract

Low-carbohydrate dietary interventions have gained scientific interest due to their potential effects on body weight, glucose regulation, and energy metabolism. Carbohydrate restriction may also influence biochemical and molecular pathways involved in nutrient sensing and metabolic regulation. The aim of the present study was to investigate physiological, metabolic, biochemical, and molecular responses to a 21-day low-carbohydrate high-protein dietary intervention in healthy young adults.

Four healthy university students (two males and two females, aged 20–26 years) participated in the study. Participants maintained carbohydrate intake below approximately 10 g per day during the intervention. Body weight, body composition, blood pressure, fasting blood glucose, and blood ketone concentrations were assessed, while clinical blood biomarkers and amino acid profiles were analyzed using external laboratory services. Gene expression of FOXO3, SIRT1, and BDNF was investigated using quantitative polymerase chain reaction (qPCR), with GAPDH used as the reference gene.

Fasting glucose concentrations were lower and blood ketone concentrations were higher during the dietary intervention compared with baseline. When the first seven intervention days were compared with the final seven days, mean fasting glucose decreased from approximately 4.41 to 4.12 mmol/L, while mean ketone concentration increased from approximately 1.24 to 2.46 mmol/L. However, neither difference reached statistical significance. Body weight decreased in all four participants, and numerical decreases were also observed in BMI, visceral fat, and blood pressure. Circulating concentrations of the branched-chain amino acids leucine, isoleucine, and valine increased numerically, although these differences were not statistically significant. Clinical biomarkers showed considerable inter-individual variability, with mixed responses in lipid and other metabolic markers. FOXO3 expression remained largely unchanged, while SIRT1 showed a numerical increase. Reliable quantitative BDNF data were not obtained and were therefore excluded from the final quantitative comparison.

Overall, the results showed metabolic adaptation to severe carbohydrate restriction, particularly through lower fasting glucose concentrations and increased ketone production. However, the small sample size, short intervention duration, absence of a control group, and inter-individual variability limit the conclusions that can be drawn. Larger and more controlled studies are required to determine whether the observed physiological, biochemical, and molecular changes are reproducible and persist during longer-term carbohydrate restriction.

Popular Scientific Summary

Low-carbohydrate diets are commonly used for weight loss and blood sugar control. When carbohydrate intake becomes very low, the body has less glucose available from food and begins to rely more on fat as an energy source. The liver also produces ketones, which can be used as an alternative source of energy. However, less is known about how short-term severe carbohydrate restriction affects other factors such as amino acids, blood biomarkers, and gene expression.

This study investigated the effects of a 21-day low-carbohydrate high-protein diet in four healthy university students aged 20–26 years. During the intervention, participants consumed less than approximately 10 g of carbohydrates per day and mainly ate protein- and fat-rich foods such as eggs, chicken, and cheese.

Fasting blood glucose and ketone concentrations were monitored throughout the study. Body weight, body composition, and blood pressure were also measured, while blood samples were used to examine clinical biomarkers, amino acid concentrations, and selected genes related to metabolism and cellular regulation.

The clearest change during the study was seen in glucose and ketone concentrations. Blood glucose generally decreased after carbohydrate restriction began, while ketone concentrations increased and remained elevated during much of the intervention. This pattern showed that the participants adapted to the very low carbohydrate intake and increased their production of ketones. However, statistical comparison of the first and final seven days of the intervention did not show significant differences in glucose or ketone concentrations.

Body weight decreased in all four participants during the 21-day period. Decreases were also observed in several other measurements, including BMI, visceral fat, and blood pressure, although these changes were not statistically significant. The concentrations of the branched-chain amino acids leucine, isoleucine, and valine increased after the intervention, but these changes were also not statistically significant. Clinical blood biomarkers varied considerably between participants.

The gene-expression results were less clear. FOXO3 remained relatively unchanged, while SIRT1 showed a numerical increase following the intervention. Reliable quantitative results were not obtained for BDNF, so this gene was not included in the final quantitative comparison.

Participants also reported temporary fatigue, weakness, and cravings during the early phase of the diet. These symptoms appeared to decrease later in the intervention, and some participants reported reduced appetite and improved energy. Since these experiences were self-reported rather than systematically measured, they should be interpreted cautiously.

Overall, the study showed that the body can adapt rapidly to severe carbohydrate restriction, particularly through increased ketone production and lower fasting glucose concentrations. However, only four participants were included and the intervention lasted for three weeks. The findings should therefore be considered preliminary, and larger and longer studies are needed before conclusions can be made about the long-term health effects of this type of diet.

1 Introduction

1.1 Modern Metabolic Disorders

Metabolic disorders have become a major global health concern over recent decades and are strongly associated with modern lifestyle patterns, including reduced physical activity, excessive caloric intake, and increased consumption of highly processed foods. Conditions such as obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular disease, and metabolic syndrome have increased substantially worldwide and continue to place significant pressure on healthcare systems. Although these disorders are multifactorial in origin, disturbances in energy metabolism, glucose regulation, lipid metabolism, and chronic low-grade inflammation are considered central contributors to disease development and progression (Hotamisligil, 2006).

Obesity is one of the most common metabolic disorders and is closely linked to several secondary complications affecting both metabolic and cardiovascular health. Excess adipose tissue accumulation, particularly visceral fat deposition, has been associated with impaired insulin sensitivity, altered lipid profiles, systemic inflammation, and increased oxidative stress. In addition to physiological alterations, obesity has also been connected to molecular and cellular changes involving mitochondrial dysfunction, inflammatory signaling pathways, and dysregulation of genes involved in metabolism and cellular survival (Gregor & Hotamisligil, 2011).

The increasing prevalence of metabolic disorders has led to growing interest in dietary strategies aimed at improving metabolic health and reducing disease risk. Among these strategies, low-carbohydrate dietary interventions have gained significant scientific attention due to their potential effects on glucose homeostasis, body composition, lipid metabolism, and ketone production. Restricting carbohydrate intake promotes a metabolic shift from glucose-dependent energy utilization toward increased fatty acid oxidation and ketone body production, resulting in altered metabolic and hormonal responses (Volek & Feinman, 2005).

In addition to systemic metabolic effects, dietary interventions may also influence molecular pathways associated with aging, neurobiology, oxidative stress regulation, and cellular adaptation. Genes such as Forkhead Box O3 (FOXO3), Sirtuin 1 (SIRT1), and Brain-Derived Neurotrophic Factor (BDNF) have been associated with cellular stress resistance, metabolic regulation, neuronal function, and longevity-related pathways (Kenyon, 2010). However, the molecular responses to short-term low-carbohydrate high-protein dietary interventions remain incompletely understood, particularly in healthy young adults.

Therefore, further investigation is needed to better understand how short-term dietary modifications influence physiological measurements, metabolic biomarkers, amino acid profiles, and gene expression patterns. Exploring these responses may provide additional insight into the metabolic and molecular adaptations associated with carbohydrate restriction and high protein intake.

1.2 Low-Carbohydrate High-Protein Diets and Metabolic Adaptation

Low-carbohydrate diets have gained increasing attention because of their potential effects on metabolic health, body composition, and glucose regulation. These diets reduce carbohydrate intake while increasing the contribution of proteins and fats to energy intake. When carbohydrate consumption is restricted, glucose availability decreases and the body gradually shifts toward increased fat utilization. As glycogen stores decline, fatty acid oxidation increases and the liver produces ketone bodies, resulting in a metabolic state known as nutritional ketosis (Masood et al., 2023).

Previous studies have reported that low-carbohydrate dietary interventions may reduce body weight, body fat, fasting glucose, and insulin levels. These effects are thought to result from reduced insulin secretion, increased fat mobilization, altered substrate utilization, and improved satiety associated with higher protein intake (Hallberg et al., 2018). High protein intake may also support muscle maintenance and metabolic regulation during carbohydrate restriction. In particular, branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, play important roles in muscle protein synthesis and nutrient-sensing pathways (Lynch & Adams, 2014).

However, individual responses to low-carbohydrate diets vary considerably. Factors such as dietary adherence, metabolic flexibility, physical activity, baseline metabolic health, and genetic differences may influence the extent of metabolic adaptation achieved (Gardner et al., 2018). In addition to physiological effects, carbohydrate restriction may influence molecular pathways involved in energy metabolism, oxidative stress regulation, and cellular adaptation, making gene expression an important area of investigation.

1.3 Amino Acid Metabolism and Branched-Chain Amino Acids

Amino acids are essential biological compounds involved in numerous physiological processes, including protein synthesis, neurotransmitter production, immune regulation, cellular signaling, and energy metabolism. Beyond their structural role in proteins, amino acids also participate in metabolic pathways that influence glucose homeostasis, nitrogen balance, oxidative stress responses, and mitochondrial function (Wu, 2009). Because dietary composition strongly affects amino acid availability and utilization, amino acid profiling has become an important tool for investigating metabolic adaptations associated with nutritional interventions.

Among the different amino acids, branched-chain amino acids (BCAAs), consisting of leucine, isoleucine, and valine, have attracted considerable scientific interest due to their involvement in muscle metabolism and nutrient signaling pathways. Unlike many other amino acids, BCAAs are primarily metabolized in skeletal muscle rather than the liver and contribute significantly to muscle protein synthesis and energy regulation (Lynch & Adams, 2014). Leucine, in particular, has been identified as an important regulator of

the mechanistic target of rapamycin (mTOR) signaling pathway, which is involved in cellular growth, nutrient sensing, and anabolic metabolism (Saxton & Sabatini, 2017).

High-protein dietary interventions may substantially alter circulating amino acid concentrations, especially levels of BCAAs and other amino acids associated with energy metabolism. Increased dietary protein intake may support muscle preservation during weight loss and contribute to increased satiety and altered substrate utilization (Paddon-Jones et al., 2008). However, elevated circulating BCAA levels have also been associated in some studies with insulin resistance, obesity, and metabolic dysfunction, suggesting that amino acid metabolism may play a complex role in metabolic health (Newgard et al., 2009).

In addition to BCAAs, several other amino acids may provide insight into metabolic adaptation during carbohydrate restriction. Glutamine and glutamate are involved in nitrogen transport and energy metabolism, while glycine participates in antioxidant defense and collagen synthesis. Taurine has been associated with mitochondrial regulation, osmoregulation, and anti-inflammatory functions, whereas tryptophan serves as a precursor for serotonin and other bioactive metabolites (Wu, 2009). Alterations in these amino acids may reflect changes in dietary intake, ketone production, protein metabolism, and overall metabolic state during dietary interventions.

Low-carbohydrate dietary interventions may also influence amino acid metabolism indirectly through altered insulin signaling, increased fatty acid oxidation, and enhanced gluconeogenesis. Reduced carbohydrate availability may shift energy metabolism toward increased utilization of fatty acids and amino acids as metabolic substrates. Furthermore, differences in dietary adherence, caloric intake, physical activity, and individual metabolic flexibility may contribute to substantial variability in amino acid responses between participants (Gannon & Nuttall, 2010).

1.4 FOXO3, SIRT1 and BDNF in Metabolic Regulation and Cellular Adaptation

Cellular adaptation to metabolic stress involves complex molecular signaling pathways that regulate energy homeostasis, oxidative stress responses, mitochondrial function, and cellular survival. Among the genes frequently associated with these processes are Forkhead Box O3 (FOXO3), Sirtuin 1 (SIRT1), and Brain-Derived Neurotrophic Factor (BDNF). These genes have attracted considerable scientific interest due to their reported involvement in metabolism, longevity-related pathways, neuroprotection, and physiological adaptation to nutritional and environmental stressors.

FOXO3 belongs to the forkhead box O family of transcription factors and plays an important role in regulating cellular stress resistance, apoptosis, antioxidant defense, and metabolic adaptation (Eijkelenboom & Burgering, 2013). FOXO3 activity is influenced by nutrient availability, insulin signaling, oxidative stress, and energy balance. Under conditions of reduced insulin signaling or metabolic stress, FOXO3 may become activated and contribute to the expression of genes involved in autophagy, antioxidant protection, DNA repair, and mitochondrial regulation (Webb & Brunet, 2014). Because low-carbohydrate dietary interventions alter glucose metabolism and

energy utilization, FOXO3 has become increasingly relevant in studies investigating metabolic adaptation and longevity-associated pathways.

Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase involved in energy metabolism, mitochondrial biogenesis, inflammation regulation, and cellular stress responses. SIRT1 has been reported to interact closely with pathways related to caloric restriction, fatty acid oxidation, and glucose homeostasis (Cantó & Auwerx, 2009). Activation of SIRT1 has been associated with improved mitochondrial function, enhanced lipid metabolism, and regulation of inflammatory signaling pathways. In addition, SIRT1 has been suggested to interact with FOXO transcription factors and other metabolic regulators involved in cellular adaptation to nutrient stress (Houtkooper et al., 2012). Consequently, dietary interventions involving carbohydrate restriction and altered energy metabolism may potentially influence SIRT1-related pathways.

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin primarily recognized for its role in neuronal survival, synaptic plasticity, and cognitive function. However, increasing evidence suggests that BDNF may also contribute to systemic energy metabolism and metabolic regulation (Mattson et al., 2004). Reduced BDNF expression has been associated with obesity, insulin resistance, neurodegenerative disease, and metabolic dysfunction. Experimental studies have suggested that dietary interventions, fasting, and exercise may influence BDNF expression through mechanisms involving neuronal energy balance and cellular stress adaptation (Marosi & Mattson, 2014).

Although these genes have individually been linked to metabolism and cellular adaptation, the effects of short-term low-carbohydrate high-protein dietary interventions on their expression remain incompletely understood, particularly in healthy young adults. Furthermore, the relationship between metabolic changes, amino acid alterations, ketone production, and gene expression responses remains an active area of investigation.

1.5 Aim of the Study

The study aimed to evaluate changes in body composition, glucose and ketone levels, clinical blood biomarkers, amino acid profiles, and the expression of selected genes associated with metabolic regulation and cellular adaptation, including FOXO3, SIRT1, and BDNF.

It was hypothesized that the dietary intervention would promote metabolic adaptations characterized by increased ketone production, reductions in body weight and glucose levels, alterations in amino acid metabolism, and measurable changes in the expression of genes associated with metabolic regulation and cellular stress responses.

2 Materials and Methods

2.1 Study Design and Participants

The present study was designed as a small exploratory pilot intervention investigating the physiological, metabolic, biochemical, and molecular effects of a short-term low-carbohydrate high-protein dietary intervention. A total of four healthy young adult participants, consisting of two males and two females between the ages of 20 to 26 years, were included in the study. All participants were university students and voluntarily participated in the intervention.

The dietary intervention lasted for 21 consecutive days. Baseline measurements were collected on same day diet started, and follow-up measurements were performed after completion of the dietary period. The study evaluated changes in physiological measurements, glucose and ketone levels, clinical blood biomarkers, amino acid profiles, and expression of selected genes associated with metabolic regulation and cellular adaptation.

All participants followed the same overall dietary approach throughout the intervention period, although exact food choices varied between individuals. Caloric intake was not standardized or intentionally restricted during the intervention. Participants were instructed to eat according to appetite while maintaining the carbohydrate restriction throughout the study period. During the intervention, participants continued their usual daily routines, including work, recreational physical activity, and sports participation.

No major medical conditions or known metabolic disorders were reported among participants prior to the intervention. No participants reported regular use of nutritional supplements during the study period. All participants successfully completed the full 21-day intervention.

2.2 Dietary Intervention

Participants were instructed to maintain carbohydrate intake below approximately 10 g per day throughout the intervention period.

Foods containing high amounts of carbohydrates and refined sugars were excluded from the diet. Participants were instructed to avoid sugar-containing foods, bread, rice, pasta, fruits, and sweetened beverages during the intervention. The dietary approach primarily consisted of protein-rich animal-based foods, with eggs, chicken, and cheese representing the most commonly consumed food items among participants. Additional dietary fats were not intentionally restricted, and consumption of fats naturally present in foods or used during cooking was permitted.

Caloric intake was not standardized or intentionally restricted during the intervention. Participants were instructed to eat according to appetite and hunger levels while maintaining carbohydrate. As a result, meal timing and total caloric consumption varied between individuals. Although one participant reported maintaining a relatively consistent caloric intake throughout the intervention, dietary intake was not formally

controlled or quantified. Despite these variations, all participants followed the same general dietary principles and completed the full intervention period.

To monitor metabolic adaptation throughout the intervention, fasting blood glucose and ketone concentrations were measured daily in the morning prior to breakfast consumption using the FreeStyle Precision Neo monitoring system (Abbott Laboratories, Abbott Park, IL, USA). Daily monitoring enabled assessment of changes in glucose regulation and ketone production during the progression of the dietary intervention.

2.3 Physiological Measurements

Physiological measurements were obtained before and after completion of the 21-day dietary intervention in order to evaluate potential changes in body composition and general physiological status. Measurements included body weight, body mass index (BMI), body fat percentage, visceral fat level, muscle mass, bone mass, basal metabolic rate (BMR), blood pressure, pulse rate, and total body water percentage.

Body composition measurements were performed using a body composition analyzer (Digital Scale BC 545 N Tanita Corporation). Participants were measured under similar conditions before and after the intervention to minimize variation associated with hydration status, food intake, and daily physiological fluctuations. Body weight was recorded in kilograms (kg), while BMI was calculated automatically by the analyzer based on height and body weight measurements.

Body fat percentage and visceral fat measurements were included to assess potential alterations in adipose tissue distribution during the intervention. Muscle mass and bone mass were also recorded to evaluate possible changes in lean tissue composition associated with carbohydrate restriction and altered dietary intake. Basal metabolic rate (BMR) was measured as an estimate of resting energy expenditure.

In addition to body composition measurements, cardiovascular-related physiological parameters including systolic blood pressure, diastolic blood pressure, and pulse rate were recorded before and after the intervention period.

All measurements were collected for each participant at baseline on same day diet started and repeated following completion of the 21-day intervention period.

2.4 Glucose and Ketone Monitoring

Measurements were performed each morning prior to breakfast consumption in order to minimize variability associated with recent food intake and daily metabolic fluctuations.

Blood glucose and ketone measurements were obtained using the FreeStyle Precision Neo monitoring system with compatible glucose and ketone test strips. Capillary blood

samples were collected through finger-prick sampling according to the manufacturer's instructions. Blood glucose concentrations were recorded in mmol/L, while ketone concentrations, primarily reflecting circulating beta-hydroxybutyrate levels, were also measured in mmol/L.

2.5 Clinical Blood Biomarker Analysis

Venous blood samples were collected under fasting conditions prior to initiation of the intervention and again following completion of the dietary period.

Blood samples were analyzed through Werlabs laboratory services, which provided standardized clinical biomarker assessment. The analyzed biomarkers included parameters associated with glucose metabolism, lipid metabolism, liver function, kidney function, inflammation, vitamin status, iron metabolism, and hormonal regulation.

Biomarkers related to glucose metabolism included fasting glucose, HbA1c, C-peptide, and insulin-related parameters. Lipid-related biomarkers included total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), and the ApoB/ApoA1 ratio. These markers were included to assess potential changes in cardiovascular and metabolic health during the intervention.

Additional biomarkers included liver enzymes such as alanine aminotransferase (ALT), inflammatory markers including high-sensitivity C-reactive protein (hs-CRP), and indicators of vitamin and mineral status including vitamin D, vitamin B12, ferritin, iron, transferrin saturation, and folic acid. Hormonal and metabolic markers such as testosterone and homocysteine were also evaluated in selected participants depending on available laboratory analyses.

2.6 Amino Acid Profiling

Amino acid analysis was conducted through GetTested laboratory services using standardized amino acid profiling methods. The analysis included measurement of 21 amino acids associated with protein metabolism, energy regulation, neurotransmitter synthesis, nitrogen balance, and metabolic adaptation. Particular emphasis was placed on branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, due to their reported involvement in nutrient signaling and metabolic regulation.

Additional amino acids analyzed included glycine, glutamine, glutamate, taurine, alanine, histidine, methionine, phenylalanine, tryptophan, proline, serine, lysine, and arginine, among others included in the amino acid profiling panel. These amino acids were evaluated to assess potential metabolic changes associated with carbohydrate restriction, increased protein intake, altered substrate utilization, and ketone production.

Because the dietary intervention primarily consisted of protein-rich foods such as eggs, chicken, and cheese, amino acid profiling was included to investigate whether increased protein intake and carbohydrate restriction influenced circulating amino acid concentrations.

2.7 RNA Extraction

Blood samples were collected in EDTA tubes and processed for RNA extraction. Approximately 1.5 mL of whole blood was mixed with 7.5 mL of Buffer EL at a 1:5 blood-to-buffer ratio for erythrocyte lysis. Samples were incubated on ice for 15 min with intermittent mixing and subsequently centrifuged at 3000 × g for 10 min at 4°C to pellet leukocytes. The supernatant was discarded, and an additional erythrocyte lysis/washing step with Buffer EL was performed to further reduce erythrocyte contamination.

Total RNA was extracted from the leukocyte pellet using the QIAamp RNA Blood Mini Kit (Qiagen, cat. no. 52304). Buffer RLT containing β-mercaptoethanol was used for cell lysis and RNase inactivation. The lysates were homogenized using QIAshredder spin columns and subsequently mixed with 70% ethanol. Samples were transferred to QIAamp spin columns, followed by washing with Buffer RW1 and Buffer RPE according to the manufacturer's protocol. RNA was subsequently eluted using RNase-free water.

RNA concentration was determined using a DS-11 Spectrophotometer (DeNovix). Extracted RNA was stored frozen until subsequent cDNA synthesis.

2.8 cDNA Synthesis

Complementary DNA (cDNA) was synthesized from the extracted RNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, cat. no. 4368814). The reverse transcription master mix contained 10× RT buffer, 25× dNTP mix, 10× random primers, and MultiScribe Reverse Transcriptase. RNase inhibitor was not included.

Each reverse transcription reaction had a total volume of 20 µL. Approximately 14.2 µL of RNA sample was combined with the reverse transcription master mix. Reaction tubes were briefly centrifuged before reverse transcription.

Reverse transcription was performed in a thermal cycler using the following program: 25°C for 10 min, 37°C for 120 min, 85°C for 5 min, followed by a hold at 4°C. Synthesized cDNA was stored frozen until qPCR analysis.

2.9 Quantitative Polymerase Chain Reaction (qPCR)

Quantitative polymerase chain reaction (qPCR) was performed to investigate the expression of FOXO3, SIRT1, and BDNF. GAPDH was used as the reference gene. qPCR was performed using the AriaMx Real-Time PCR System (Agilent Technologies) with Fast SYBR Green Master Mix (Thermo Fisher Scientific, cat. no. 4385612).

Each qPCR reaction had a total volume of 10 µL, consisting of 5 µL Fast SYBR Green Master Mix (1×), 3 µL primer mixture (1.5 µM of each forward and reverse primer), 1 µL cDNA (1 ng/µL), and 1 µL purified water.

Thermal cycling consisted of an initial activation step at 95°C for 3 min, followed by 50 amplification cycles of denaturation at 95°C for 5 s and annealing/extension at 60°C for 10 s.

Relative gene expression was determined using the relative standard curve method. Standard curves were generated using four-fold serial dilutions of pooled cDNA prepared from participant samples. Relative quantities of the target genes were calculated from the standard curves using AriaMx software. Target-gene expression was normalized to GAPDH, and normalized expression values were compared between the pre- and post-intervention samples.

Table 1. Primer sequences used for qPCR analysis (5′–3′).

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
Foxo3	CCCAGCCTAACCAGGGAAGT	AGCGCCCTGGGTTTGG
SIRT1	TACGACGAAGACGACGACGA	CGCCGCCGCCGCCTCTTCC
BDNF	CACTGGCTGACACTTTTGAGCAC	AGCCTTCATGCAACCGAAGT
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

2.10 Statistical Analysis

Data from physiological measurements, clinical blood biomarkers, amino acid profiling, and gene-expression analyses were organized and analyzed using Microsoft Excel. Results are presented as mean ± standard deviation (SD), unless otherwise stated.

For variables measured once before and once after the dietary intervention, paired participant-level comparisons were performed using the Wilcoxon signed-rank test. A non-parametric approach was used because of the small sample size ($n = 4$) and paired study design. Statistical significance was defined as $p < 0.05$. Given the small sample size and exploratory nature of the study, the direction of change and inter-individual variability were also considered when evaluating the results.

Fasting blood glucose and ketone concentrations were measured repeatedly during the baseline and intervention periods and were therefore evaluated separately from variables measured only before and after the intervention. Daily concentrations were summarized as group means ± SD and plotted over time. To evaluate changes during the dietary intervention, mean concentrations during the first seven intervention days (days 1–7) and the final seven intervention days (days 15–21) were calculated separately for each participant and compared using the Wilcoxon signed-rank test.

For gene-expression analysis, relative quantities obtained from the standard-curve-based qPCR analysis were normalized to GAPDH by dividing the quantity of each target gene by the corresponding GAPDH quantity. Normalized FOXO3 and SIRT1 expression values were summarized as mean ± SD before and after the intervention. BDNF was not included in the final quantitative analysis because reliable quantitative data were not available.

3 Results

3.1 Baseline Characteristics and Physiological Measurements

A total of four healthy young adults participated in the 21-day dietary intervention study. Baseline physiological measurements were collected prior to initiation of the intervention and repeated following completion of the dietary period. Measurements included body weight, body mass index (BMI), body fat percentage, body water percentage, muscle mass, bone mass, basal metabolic rate (BMR), visceral fat, blood pressure, and pulse rate.

Overall, participants demonstrated reductions in body weight, BMI, body fat percentage, and visceral fat measurements following the 21-day dietary intervention, although the magnitude of change varied between individuals. Average body weight decreased across participants during the intervention period, accompanied by reductions in BMI values. Body fat percentage demonstrated modest decreases in most participants, while body water percentage generally increased following the intervention. Mean body weight demonstrated a downward trend following the intervention period. However, Wilcoxon signed-rank analysis did not demonstrate statistical significance ($p > 0.05$). In contrast, slight reductions in muscle mass and basal metabolic rate were observed in several participants compared to baseline measurements (Figure 1).

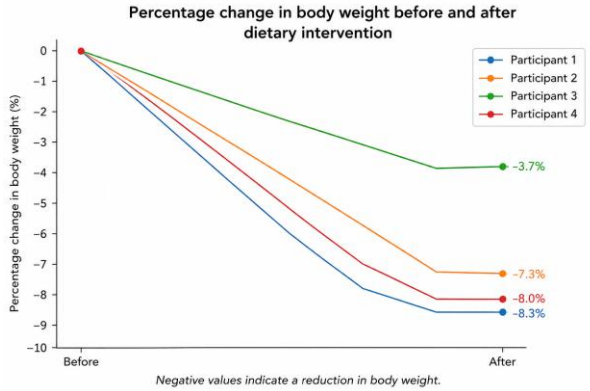

Figure 1. Body weight before and after dietary intervention.

3.2 Glucose and Ketone Responses

Fasting blood glucose and blood ketone concentrations changed during the 21-day dietary intervention (Figure 2). During the baseline period, mean glucose concentrations

remained relatively stable at around 5 mmol/L, while ketone concentrations remained low.

Following the start of the dietary intervention, mean fasting glucose decreased and remained generally below baseline levels throughout the intervention. The mean participant-level glucose concentration was approximately 5.24 mmol/L during the baseline period and 4.24 mmol/L during the intervention period.

In contrast, blood ketone concentrations increased after the start of carbohydrate restriction. The mean participant-level ketone concentration was approximately 0.08 mmol/L during the baseline period and increased to approximately 2.04 mmol/L during the intervention. The increase was most apparent during the first week, after which mean ketone concentrations remained elevated. Considerable variation between participants was observed, particularly for ketone concentrations during the later stages of the intervention.

To assess changes over the course of the intervention, mean concentrations during the first seven intervention days were compared with those during the final seven intervention days. Mean fasting glucose decreased from approximately 4.41 mmol/L during days 1–7 to 4.12 mmol/L during days 15–21. Mean blood ketone concentration increased from approximately 1.24 mmol/L to 2.46 mmol/L over the same periods. However, neither the change in glucose ($p = 0.375$) nor the change in ketone concentration ($p = 0.250$) reached statistical significance using the Wilcoxon signed-rank test.

Overall, the daily measurements showed a pattern of lower fasting glucose concentrations and higher blood ketone concentrations following the start of the dietary intervention (Figure 2).

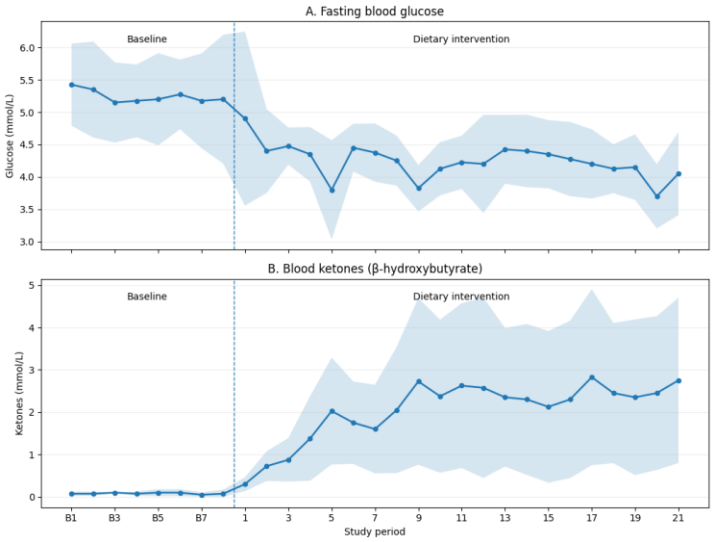

Figure 2. Changes in fasting blood glucose and blood β-hydroxybutyrate concentrations during the baseline and 21-day dietary intervention periods (n = 4). (A) Mean fasting blood glucose concentration and (B) mean blood β-hydroxybutyrate concentration. Points represent group means and shaded areas represent standard deviation (SD). The dashed vertical line indicates the start of the dietary intervention.

3.3 Physiological and Body Composition Changes Following the Intervention

Physiological measurements collected before and after the dietary intervention demonstrated notable changes in body composition and metabolic-related parameters following the 21-day low-carbohydrate high-protein dietary intervention.

Following the 21-day dietary intervention, mean body weight decreased from 84.0 ± 14.1 kg to 78.2 ± 12.0 kg, corresponding to a mean reduction of 5.9 kg. Mean BMI decreased from 26.8 ± 5.9 to 24.8 ± 4.9 kg/m², while body fat percentage decreased from 30.5 ± 5.8% to 28.5 ± 5.9%. Mean visceral fat level decreased from 5.6 ± 3.3 to 4.3 ± 2.6. In contrast, mean body water increased from 50.4 ± 3.6% to 51.6 ± 3.5%. Mean muscle mass decreased from 55.9 ± 13.2 kg to 53.5 ± 11.6 kg, and estimated basal metabolic rate decreased from 1801 ± 392 to 1714 ± 339 kcal/day. None of these paired changes reached statistical significance using the Wilcoxon signed-rank test (all $p = 0.125$).

Mean systolic blood pressure decreased from 118.8 ± 11.3 mmHg before the intervention to 109.0 ± 10.1 mmHg after the intervention, while mean diastolic blood pressure decreased from 82.6 ± 13.0 to 73.8 ± 11.8 mmHg. These changes did not reach statistical significance using the Wilcoxon signed-rank test ($p = 0.125$ for both).

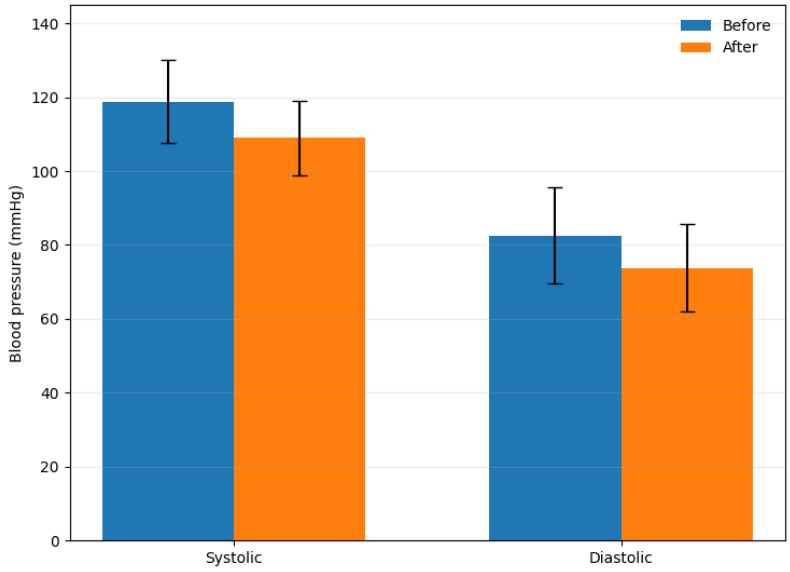

Figure 3. Mean systolic and diastolic blood pressure before and after the 21-day dietary intervention in four participants. Error bars represent SD. Wilcoxon signed-rank testing showed no statistically significant pre- and post-intervention differences.

3.4 Clinical Blood Biomarker Changes

Clinical blood biomarkers showed considerable inter-individual variability following the dietary intervention (Figure 4). Mean total cholesterol changed from 5.55 ± 2.60 mmol/L

before the intervention to 5.38 ± 1.89 mmol/L afterwards, while mean LDL cholesterol remained relatively unchanged, changing from 4.03 ± 2.08 to 3.98 ± 1.53 mmol/L.

Mean HDL cholesterol decreased from 1.27 ± 0.40 to 0.96 ± 0.40 mmol/L, whereas mean triglyceride concentration increased from 1.11 ± 0.69 to 1.53 ± 0.60 mmol/L. None of the lipid-related changes reached statistical significance using the Wilcoxon signed-rank test ($p > 0.05$).

Mean hs-CRP decreased from 6.81 ± 11.41 to 2.98 ± 3.69 mg/L, although the group mean was strongly influenced by a high baseline value in one participant. Mean vitamin D concentration remained relatively unchanged (48.3 ± 37.4 vs. 47.8 ± 38.7 nmol/L), while ferritin increased from 48.8 ± 29.2 to 70.5 ± 46.4 µg/L and HbA1c decreased from 34.8 ± 2.6 to 32.8 ± 3.1 mmol/mol. None of these paired changes reached statistical significance ($p > 0.05$).

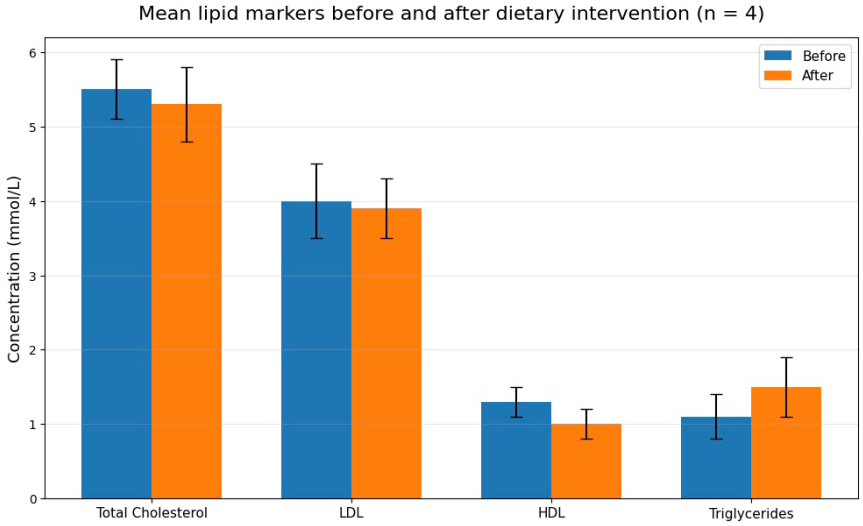

Figure 4. Mean lipid biomarker concentrations including total cholesterol, LDL, HDL, and triglycerides.

3.5 Amino Acid Profile Changes

Branched-chain amino acid concentrations increased following the dietary intervention (Figure 5).

Mean isoleucine concentration increased from 50.5 ± 11.5 µmol/L before the intervention to 72.3 ± 27.7 µmol/L after the intervention. Mean leucine concentration increased from 91.7 ± 22.3 to 117.6 ± 42.4 µmol/L, while mean valine increased from 176.1 ± 33.0 to 234.8 ± 46.8 µmol/L.

All four participants showed an increase in isoleucine, leucine, and valine following the dietary intervention. Despite the consistent direction of change, none of the differences

reached statistical significance using the Wilcoxon signed-rank test ($p = 0.125$ for isoleucine, leucine, and valine).

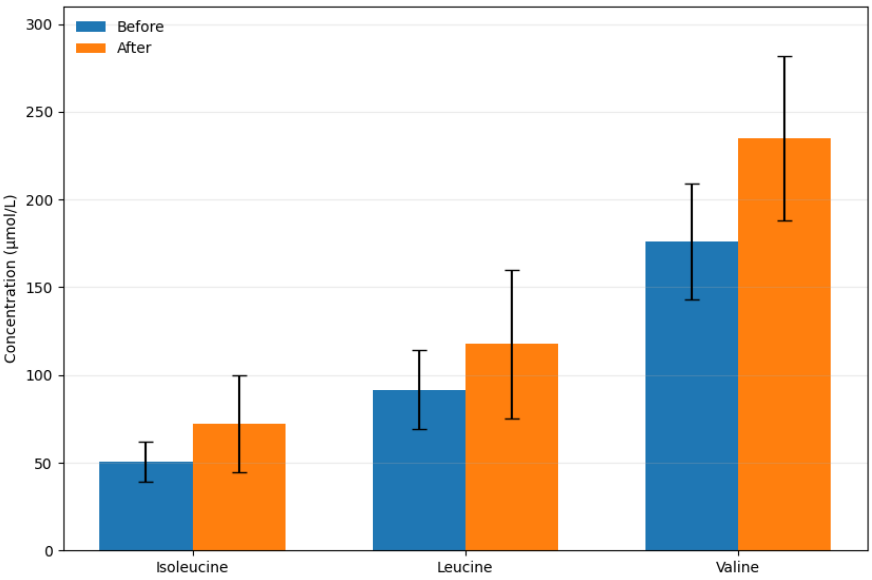

Figure 5. Mean circulating isoleucine, leucine, and valine concentrations before and after the 21-day dietary intervention in four participants. Error bars represent SD. Wilcoxon signed-rank testing showed no statistically significant pre- and post-intervention differences.

3.6 Gene Expression Analysis

qPCR analysis was performed to investigate expression of genes associated with metabolic regulation and cellular adaptation. GAPDH was used as the reference gene for normalization.

Based on the available quantitative results, mean normalized FOXO3 expression remained almost unchanged following the dietary intervention. Mean FOXO3 expression was approximately 0.60 ± 0.26 before the intervention and 0.61 ± 0.16 after the intervention.

Mean normalized SIRT1 expression showed a numerical increase, from approximately 0.66 ± 0.46 before the intervention to 0.87 ± 0.42 after the intervention. Considerable variation was present between participants, particularly for SIRT1.

No formal inferential statistical comparison was reported for FOXO3 or SIRT1 in the final analysis. FOXO3 remained relatively stable at group level, whereas SIRT1 showed a numerical increase following the intervention.

BDNF was included as an original qPCR target; however, sufficiently reliable quantitative BDNF data were not obtained for inclusion in the final analysis. BDNF was therefore excluded from Figure 6 and is considered a technical limitation of the molecular analysis.

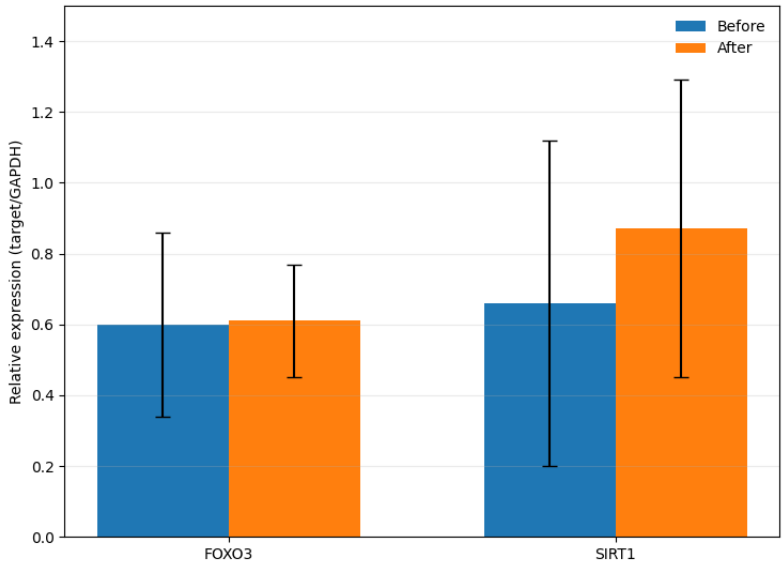

Figure 6. GAPDH-normalized FOXO3 and SIRT1 expression before and after the 21-day dietary intervention. Values represent mean ± SD for the available participant measurements.

4 Discussion

4.1 Overview of Main Findings

The present study investigated physiological, metabolic, biochemical, and molecular responses to a 21-day low-carbohydrate high-protein dietary intervention in four healthy young adults. The clearest response to the intervention was the change in circulating glucose and ketone concentrations. Fasting glucose was lower during the intervention than during baseline, while ketone concentrations increased markedly following carbohydrate restriction. Body weight decreased in all four participants, while changes were also observed in body composition and blood pressure. However, the small sample size and lack of statistically significant differences for most outcomes require these findings to be interpreted as descriptive observations rather than evidence of a treatment effect.

The biochemical analyses showed variable responses. Circulating concentrations of the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine increased numerically following the intervention, whereas lipid biomarkers showed mixed responses. FOXO3 expression remained largely unchanged, while SIRT1 showed a numerical increase after the intervention. Taken together, the results indicate that substantial metabolic adaptation occurred during carbohydrate restriction, but the physiological, biochemical, and molecular responses varied considerably between participants.

4.2 Metabolic Adaptation and Ketosis

The increase in blood ketone concentrations provides the clearest indication that participants adapted metabolically to the very low carbohydrate intake. Before the intervention, ketone concentrations were low, whereas they increased rapidly after carbohydrate restriction and remained elevated during much of the intervention. This response is consistent with the expected metabolic consequences of carbohydrate restriction, where reduced carbohydrate availability and depletion of glycogen stores increase reliance on fatty acid oxidation and hepatic ketone production (Volek & Feinman, 2005).

Fasting glucose also decreased following the start of the intervention. Mean glucose was approximately 5.24 mmol/L during baseline and 4.24 mmol/L across the intervention period. When the first seven intervention days were compared with the final seven days, glucose decreased from approximately 4.41 to 4.12 mmol/L, whereas ketones increased from approximately 1.24 to 2.46 mmol/L. These differences were not statistically significant using the Wilcoxon signed-rank test ($p = 0.375$ for glucose and $p = 0.250$ for ketones). Therefore, the data demonstrate a clear temporal pattern but do not provide sufficient statistical evidence to establish a difference between the early and late intervention periods.

The metabolic pattern is nevertheless biologically consistent with carbohydrate restriction. When dietary carbohydrate availability is low, hepatic glycogen availability decreases and fatty acids become increasingly important as energy substrates, resulting in increased ketogenesis (Volek & Feinman, 2005). Glucose concentrations can remain within the physiological range because endogenous glucose production, particularly gluconeogenesis, continues to supply glucose to tissues that require it.

Considerable variation in ketone concentrations was observed between participants. This may reflect differences in dietary intake, energy balance, physical activity, metabolic flexibility, and individual responses to carbohydrate restriction. Because food intake and physical activity were not standardized, it is not possible to determine which of these factors contributed most strongly to the observed variation.

4.3 Physiological and Body Composition Changes

The physiological measurements obtained during the study demonstrated changes in body composition and cardiovascular-related parameters following the 21-day low-carbohydrate high-protein dietary intervention. The most notable findings were reductions in body weight, BMI, visceral fat, and blood pressure, suggesting metabolic adaptation during the intervention period.

The reduction in body weight likely resulted from several interacting mechanisms, including glycogen depletion, increased lipolysis, reduced insulin signaling, appetite-related reductions in caloric intake, and greater reliance on fatty acids as energy substrates. Similar reductions in body weight have been reported in previous low-carbohydrate dietary studies (Hallberg et al., 2018).

Reductions in BMI and visceral fat may indicate improvements in body composition and metabolic health. Visceral adipose tissue is closely associated with insulin resistance,

inflammation, and cardiometabolic risk, making reductions in visceral fat generally considered beneficial (Gregor & Hotamisligil, 2011).

Decreases in measured muscle mass and basal metabolic rate (BMR) were also observed. These findings may partly reflect glycogen depletion and the associated loss of intracellular water, since glycogen is stored together with water in skeletal muscle. Reduced caloric intake and overall body mass may have further contributed to the decline in BMR during the intervention.

Participants additionally demonstrated reductions in systolic and diastolic blood pressure. These findings are consistent with previous reports suggesting that carbohydrate restriction may influence blood pressure through changes in body weight, insulin levels, sodium balance, and overall metabolic status. However, because blood pressure can be affected by hydration status and daily physiological variation, these findings should be interpreted cautiously.

Several participants also reported reduced appetite following the initial adaptation phase. This may be related to increased protein intake, ketone production, and improved satiety, which could contribute to spontaneous reductions in caloric intake despite the absence of intentional calorie restriction.

Overall, the physiological findings suggest that short-term low-carbohydrate high-protein dietary interventions can induce measurable changes in body composition and metabolic health. However, the small sample size and considerable inter-individual variability warrant cautious interpretation of the results.

4.4 Amino Acid Metabolism and Protein Intake

Mean concentrations of leucine, isoleucine, and valine increased following the intervention, although none of the changes reached statistical significance. Because participants consumed a diet rich in protein-containing foods such as eggs, chicken, and cheese, the numerical increase in circulating BCAAs may reflect increased dietary protein intake. BCAAs are involved in skeletal muscle metabolism and nutrient signaling, with leucine in particular contributing to regulation of the mTOR pathway (Saxton & Sabatini, 2017).

However, increased circulating BCAA concentrations should not automatically be interpreted as beneficial. Elevated BCAA concentrations have also been associated with insulin resistance and metabolic dysfunction in some populations (Newgard et al., 2009). In the present study, the intervention was short and involved healthy young adults, so the observed numerical increases are more appropriately described as changes associated with the dietary intervention rather than indicators of improved or impaired metabolic health.

The gene-expression analysis provided more limited evidence of molecular adaptation. FOXO3 expression remained essentially unchanged, increasing only from approximately 0.60 to 0.61 after GAPDH normalization. SIRT1 showed a larger numerical increase, from approximately 0.66 to 0.87. However, these results should not be interpreted as evidence that the intervention altered transcription of these genes because of the small sample size and technical variability associated with the qPCR analysis.

SIRT1 is involved in cellular energy regulation and has been associated with responses to altered nutrient availability (Cantó & Auwerx, 2009), while FOXO3 participates in pathways related to cellular stress responses and metabolic regulation (Eijkelenboom & Burgering, 2013). The numerical SIRT1 increase is therefore of exploratory interest, but confirmation would require a larger study with more robust molecular measurements. Reliable quantitative BDNF data were not obtained and BDNF was therefore excluded from the final quantitative comparison.

4.5 Strengths and Limitations

The main strength of the study was the combination of several types of measurements within the same dietary intervention. Daily glucose and ketone monitoring provided information about the temporal metabolic response, while physiological measurements, clinical biomarkers, amino acid profiling, and gene-expression analysis allowed the intervention to be examined at several biological levels.

The most important limitation was the small sample size (n = 4), which resulted in low statistical power and limits generalization of the findings. The absence of a control group also means that changes observed over the 21-day period cannot be attributed exclusively to the dietary intervention. In addition, caloric intake, exact protein and fat intake, meal timing, and physical activity were not standardized or quantitatively monitored. These factors may have contributed to the considerable inter-individual variation.

The short intervention duration further limits conclusions regarding long-term effects. Changes in body weight and bioelectrical-impedance measurements may have been influenced by glycogen and hydration changes rather than changes in tissue mass alone. The qPCR experiments also involved technical challenges, including inconsistent amplification and non-specific amplification in some reactions, which reduced confidence in the molecular results. These limitations are particularly important when interpreting the SIRT1 and FOXO3 findings.

4.6 Conclusion and Future Perspectives

The 21-day low-carbohydrate high-protein intervention was accompanied by lower fasting glucose concentrations, increased blood ketone concentrations, reductions in body weight, and numerical changes in several physiological and biochemical measurements. The glucose and ketone time course was consistent with metabolic adaptation to severe carbohydrate restriction, although comparisons between the first and final seven intervention days were not statistically significant. BCAA concentrations increased numerically, FOXO3 expression remained largely unchanged, and SIRT1 showed an exploratory upward trend.

Because the study included only four participants, lacked a control group, and involved a short and incompletely standardized dietary intervention, causal or long-term conclusions cannot be drawn. Future studies should include larger participant groups, longer intervention periods, controlled or accurately recorded dietary intake, and standardized physical activity. Improved qPCR replication and additional molecular measurements would also be required to determine whether the metabolic changes

associated with carbohydrate restriction are accompanied by reproducible changes in gene expression.

5 Declaration of AI and AI-Assisted Technologies

During the preparation and revision of this thesis, ChatGPT (OpenAI) was used extensively as an AI-assisted tool. Its use included language and grammar correction, restructuring and rewriting of text, improvement of scientific writing and coherence, organization of sections, and assistance in revising the thesis in response to supervisor and examiner feedback. ChatGPT was also used to help clarify scientific and statistical concepts, check statistical calculations, and improve the presentation and interpretation of the results and discussion.

The study design, experimental procedures, laboratory work, and primary data collection were carried out by the author. All experimental data presented in the thesis originate from the study and were not generated or fabricated using AI. ChatGPT was used to assist in the analysis, interpretation, presentation, and written discussion of these data. The author reviewed the AI-assisted content and takes full responsibility for the final analyses, interpretations, conclusions, and submitted text.

Ethical Aspects and Societal Impact of the Research

Several ethical considerations were relevant to the present study. All participants took part voluntarily and were informed about the purpose of the study, the dietary intervention, and the measurements that would be performed. Participants were free to withdraw from the study at any time without consequences. Personal information and biological data were handled confidentially, and participant identities were anonymized during data analysis and presentation of results. Because blood sampling and dietary modification were involved, attention was given to participant safety throughout the intervention period.

The dietary intervention required participants to substantially restrict carbohydrate intake while increasing protein consumption. Although low-carbohydrate diets are widely used and generally considered safe for healthy individuals over short periods, such interventions may not be suitable for everyone. Individuals with certain medical conditions, metabolic disorders, kidney disease, or specific nutritional requirements may respond differently. Therefore, dietary recommendations should always be individualized and based on scientific evidence and professional guidance.

From a societal perspective, research on dietary interventions is highly relevant because obesity, type 2 diabetes, cardiovascular disease, and other metabolic disorders represent major public health challenges worldwide. Improved understanding of how dietary composition influences metabolism, body composition, and molecular pathways may contribute to the development of more effective nutritional strategies for disease prevention and health promotion.

At the same time, findings from small exploratory studies should be interpreted cautiously. There is a risk that results may be oversimplified or generalized beyond the studied population. Consequently, the findings of the present study should not be considered universal dietary recommendations but rather as preliminary observations that contribute to the growing body of knowledge regarding carbohydrate restriction and metabolic adaptation. Future research involving larger and more diverse populations is necessary before broader conclusions can be drawn.

Overall, the study was conducted with respect for participant welfare, privacy, and scientific integrity, while addressing a research topic with potential relevance for both public health and nutritional science.

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Appendix A

Table A. Mean fasting glucose and ketone concentrations before and after the dietary intervention across all participant (n=4).

Participant	Mean baseline glucose (mmol/L)	Mean post-intervention glucose (mmol/L)	Mean baseline ketones (mmol/L)	Mean post-intervention ketones (mmol/L)
Participant A	4.59	3.77	0.13	3.41
Participant B	6.18	4.60	0.08	0.98
Participant C	5.25	4.03	0.10	3.00
Participant D	4.96	4.56	0.03	0.78

Appendix B

Table B. Mean physiological and body composition measurements before and after dietary intervention across all participants (n=4).

Parameter	Before intervention (Mean)	After intervention (Mean)
Body weight (Kg)	84.0	78.1
BMI	26.8	24.8
Body fat (%)	30.4	28.5
Water (%)	50.3	51.5
Muscle mass (Kg)	55.9	53.4
Basal metabolic rate (Kcal)	1801.0	1713.7
Metabolic age	38.0	35.5
Visceral fat level	5.6	4.3
Blood pressure	118.7	109.0
Pulse	81	79