PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

PEPTÍDEOS DERIVADOS DAS MITOCÔNDRIAS, GLICEMIA E NÍVEIS DE HEMOGLOBINA GLICADA EM HOMENS COM DIABETES MELLITUS TIPO 2, ATEROSCLEROSE OU HIPERTENSÃO: ESTUDO TRANSVERSAL

MITOCHONDRIAL-DERIVED PEPTIDES, BLOOD GLUCOSE, AND GLYCATED HEMOGLOBIN LEVELS IN MEN WITH TYPE 2 DIABETES, ATHEROSCLEROSIS, OR HYPERTENSION: A CROSS-SECTIONAL STUDY

قيم الببتيدات المشتقة من الميتوكوندريا، وسكر الدم، والهيموغلوبين السكري لدى الرجال المصابين بداء السكري من النوع الثاني أو تصلّب الشرايين أو ارتفاع ضغط الدم :دراسة مقطعية

Inass Majid Mozan
[1] Department of Biology, College of Science, University of Misan, Maysan, Iraq.
ORCID: https://orcid.org/0009-0004-7481-301X

Zainab Abduljabber Ridha Al-Ali *
Department of Biology, College of Science, University of Misan, Maysan, Iraq.
ORCID: https://orcid.org/0000-0001-9621-9952

Redha Alwan Hasan
College of Medicine, University of Misan, Maysan, Iraq. ORCID: https://orcid.org/0000-0002-2432-1648

** Corresponding author*
e-mail: zainab-alali@uomisan.edu.iq

Received March 29; received in revised form 01 July 2026; accepted 15 July 2026

RESUMO

Introdução: Os peptídeos derivados das mitocôndrias, incluindo a humanina e o peptídeo codificado pelo quadro aberto de leitura mitocondrial do RNA ribossômico 12S tipo c (MOTS-c), participam da resposta ao estresse celular e da regulação metabólica. **Objetivo**: Comparar as concentrações séricas de humanina e MOTS-c, hemoglobina glicada, glicemia de jejum, insulina e resistência à insulina entre homens saudáveis e homens com diabetes mellitus tipo 2, aterosclerose ou hipertensão. **Métodos**: Este estudo transversal incluiu 88 homens de 40–60 anos, recrutados entre dezembro de 2024 e outubro de 2025 e distribuídos em quatro grupos (n = 22 por grupo). A hemoglobina glicada foi medida em sangue total; glicose, insulina, humanina e MOTS-c foram determinadas no soro. A resistência à insulina foi estimada pelo índice HOMA-IR. As comparações foram realizadas por análise de variância de uma via seguida do teste post hoc da diferença mínima significativa. **Resultados**: A humanina não diferiu entre os grupos ($P > 0,05$). O MOTS-c foi significativamente menor nos três grupos de pacientes do que no controle ($P < 0,001$). A hemoglobina glicada, a glicemia de jejum e o HOMA-IR foram mais elevados no grupo com diabetes tipo 2; as concentrações de insulina também diferiram significativamente entre os grupos. **Discussão**: A redução do MOTS-c pode estar associada a alterações da homeostase mitocondrial e metabólica, enquanto a humanina circulante não diferenciou os grupos. **Conclusões**: Baixas concentrações de MOTS-c estiveram associadas a diabetes tipo 2, aterosclerose e hipertensão; entretanto, o desenho transversal, a amostra limitada e as análises não ajustadas impedem inferências causais e generalizações amplas.

Palavras-chave: *humanina; MOTS-c; peptídeos derivados das mitocôndrias; resistência à insulina; hemoglobina glicada*

ABSTRACT

Background: Mitochondrial-derived peptides, including humanin and mitochondrial open reading frame of the 12S ribosomal RNA type-c (MOTS-c), are bioactive microproteins involved in cellular stress responses and metabolic regulation. **Aim:** To compare serum humanin and MOTS-c, glycated hemoglobin, fasting blood glucose, insulin, and insulin resistance among healthy men and men with type 2 diabetes mellitus, atherosclerosis, or hypertension. **Methods:** This cross-sectional study included 88 men aged 40–60 years who were recruited between December 2024 and October 2025 and assigned to four groups (n = 22 each). Glycated hemoglobin was measured in whole blood; glucose, insulin, humanin, and MOTS-c were measured

DOI: 10.52571/PTQ.v23.n53.2026_06_MOZAN_pgs_64_75.pdf

in serum. Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR). Group differences were evaluated using one-way analysis of variance followed by the least significant difference post hoc test. **Results:** Humanin did not differ among groups [$F(3, 84) = 1.013$, $P = 0.391$]. MOTS-c was markedly lower in all patient groups than in controls [$F(3, 84) = 113.339$, $P < 0.001$]. Glycated hemoglobin, fasting glucose, and HOMA-IR were highest in the type 2 diabetes group, and insulin concentrations differed significantly among groups. **Discussion:** Reduced circulating MOTS-c may reflect altered mitochondrial and metabolic homeostasis, whereas circulating humanin did not discriminate among the groups. **Conclusions:** Lower MOTS-c concentrations were associated with type 2 diabetes, atherosclerosis, and hypertension. Because recruitment was site-based and the analyses were unadjusted, the findings are exploratory and do not establish causality.

Keywords: *mitochondrial-derived peptides; humanin; MOTS-c; insulin resistance; glycated hemoglobin*

الملخص:

الخلفية: تُعدّ الببتيدات المشتقة من الميتوكوندريا، ومنها الهيومانين والببتيد MOTS-c، بروتينات دقيقة فعّالة حيوياً تسهم في الاستجابة للإجهاد الخلوي وتنظيم الاستقلاب. **الهدف**: مقارنة تراكيز الهيومانين وMOTS-c في المصل، والهيموغلوبين السكري، وسكر الدم الصائم، والإنسولين، ومقاومة الإنسولين بين رجال أصحاء ورجال مصابين بداء السكري من النوع الثاني أو تصلّب الشرايين أو ارتفاع ضغط الدم. **طرائق العمل**: شملت هذه الدراسة المقطعية 88 رجلاً تراوحت أعمارهم بين 40 و60 عاماً، جرى استقطابهم خلال المدة من كانون الأول/ديسمبر 2024 إلى تشرين الأول/أكتوبر 2025، وقُسّموا إلى أربع مجموعات بواقع 22 مشاركاً لكل مجموعة. قيس الهيموغلوبين السكري في الدم الكامل، في حين قيس كل من الجلوكوز والإنسولين والهيومانين وMOTS-c في المصل، وحُسبت مقاومة الإنسولين باستعمال مؤشر HOMA-IR. وقورنت المجموعات بتحليل التباين الأحادي متبوعاً باختبار أقل فرق معنوي. **النتائج**: لم تختلف تراكيز الهيومانين معنوياً بين المجموعات ($P > 0.05$)، بينما انخفضت تراكيز MOTS-c معنوياً في مجموعات المرضى الثلاث مقارنةً بالمجموعة الضابطة ($P < 0.001$). وكانت قيم الهيموغلوبين السكري وسكر الدم الصائم وHOMA-IR الأعلى في مجموعة السكري من النوع الثاني، كما اختلفت تراكيز الإنسولين معنوياً بين المجموعات. **المناقشة**: قد يرتبط انخفاض MOTS-c باضطراب الاتزان الميتوكوندري والاستقلابي، في حين لم تميّز تراكيز الهيومانين الدورية بين المجموعات. **الاستنتاجات**: ارتبط انخفاض MOTS-c بداء السكري من النوع الثاني وتصلّب الشرايين وارتفاع ضغط الدم، إلا أن التصميم المقطعي وحجم العينة المحدود والتحليلات غير المعدّلة لا تسمح بإثبات السببية أو التعميم الواسع.

الكلمات المفتاحية: *الببتيدات المشتقة من الميتوكوندريا؛ الهيومانين؛MOTS-c ؛ مقاومة الإنسولين؛ الهيموغلوبين السكري.*

1. INTRODUCTION:

Mitochondria coordinate cellular bioenergetics, redox balance, apoptosis, and stress signaling. Beyond adenosine triphosphate production, mitochondrial DNA encodes small biologically active peptides that participate in inter-organelle and systemic signaling (Saminathan *et al*., 2022; Clemente-Suárez *et al*., 2023). These mitochondrial-derived peptides include humanin, mitochondrial open reading frame of the 12S ribosomal RNA type-c (MOTS-c), small humanin-like peptides 1–6, SHMOOSE, and Gau (Saghatelian and Couso, 2015; Kumagai *et al*., 2023).

Humanin is encoded within the mitochondrial MT-RNR2 region and was originally identified through its neuroprotective activity (Hashimoto *et al*., 2001). It has antiapoptotic, cytoprotective, and metabolic effects, although circulating concentrations may vary with age, tissue activity, and disease state (Salemi *et al*., 2020; Zuccato *et al*., 2019; Coradduzza *et al*., 2023). MOTS-c is encoded within MT-RNR1 and can modulate cellular metabolism through pathways that include AMP-activated protein kinase; experimental evidence links it to improved glucose uptake, insulin sensitivity, and energy homeostasis (Lee *et al*., 2015; Kim *et al*., 2019; Merry *et al*., 2020).

Type 2 diabetes mellitus is characterized by insulin resistance, compensatory hyperinsulinemia, and progressive β-cell dysfunction (Schleicher *et al*., 2022). Mitochondrial-derived peptides have been implicated in pancreatic β-cell survival and metabolic regulation (Hoang *et al*., 2010). In vascular disease, humanin may protect endothelial cells from oxidized low-density lipoprotein-related injury, whereas MOTS-c has been associated with metabolic and vascular homeostasis (Evangeline and Iyer, 2021; Zhu *et al*., 2017; Li *et al*., 2019). Clinical studies have reported altered circulating humanin or MOTS-c in diabetes and coronary artery disease, but findings are not uniform across populations and clinical phenotypes. Hypertension frequently coexists with dysglycemia and insulin

resistance, and elevated fasting glucose has been associated with a subsequent risk of hypertension, particularly in men (Ahn *et al*., 2021). Because mitochondrial stress signaling contributes to metabolic and vascular homeostasis, altered circulating mitochondrial-derived peptides may also accompany hypertensive cardiometabolic phenotypes, although direct clinical evidence remains limited (Savvopoulos *et al*., 2024).

Direct comparisons of both humanin and MOTS-c across type 2 diabetes, atherosclerosis, and hypertension using the same sampling and assay framework remain limited, particularly in Iraqi men. We hypothesized that circulating mitochondrial-derived peptides would differ between healthy controls and the three patient groups and would parallel disturbances in glycemic control and insulin resistance.

1.1. Aim

The objective of this study was to compare serum humanin and MOTS-c concentrations, glycated hemoglobin, fasting blood glucose, insulin, and homeostatic model assessment of insulin resistance (HOMA-IR) among healthy men and men with type 2 diabetes mellitus, atherosclerosis, or hypertension

2. MATERIALS AND METHODS:

2.1. Study Population

This cross-sectional study was conducted at the Center for Diabetes and Endocrinology, the Maysan Center for Cardiac Diseases and Surgery, Al-Taif Laboratory, and selected private clinics in Maysan, Iraq, from December 2024 to October 2025. Eligibility was restricted to men aged 40–60 years who belonged to one of four predefined clinical categories and provided written informed consent. The final analyzed sample comprised 88 participants: healthy controls (group A), type 2 diabetes mellitus (group B), atherosclerosis (group C), and hypertension (group D), with 22 participants in each group. All 88 enrolled participants were included in the reported analyses. No formal a priori power calculation was performed; the sample size reflected recruitment feasibility during the prespecified period, and the study should therefore be regarded as exploratory.

Patient-group diagnoses were established by specialist physicians using medical history, clinical findings, and available medical records at the participating centers. Medication histories were recorded descriptively but were not used for stratification or covariate adjustment. Standardized eligibility, fasting blood collection, and laboratory processing procedures were applied to reduce measurement variability; however, the site-based non-random recruitment strategy may have introduced selection bias.

2.2. Blood Samples

Six milliliters of venous blood were collected from each participant after an 8-hour fast between 09:00 and 11:00. Two milliliters were placed in an ethylenediaminetetraacetic acid tube for glycated hemoglobin determination using the Huma Meter A1C reagent system (Human, Germany) according to Nathan *et al*. (2008). The remaining 4 mL were placed in a gel tube, allowed to clot for 15 minutes, and centrifuged at 3,000 rpm for 5 minutes. Serum glucose was measured using a BioSystems A15 automated spectrophotometer (Spain) according to standard clinical chemistry procedures. Residual serum was stored at –20 °C until analysis. Humanin and MOTS-c were measured using immunoassay kits supplied by MyBioSource (USA) and the ELISYS Uno system (Human, Germany), respectively. Insulin was measured using the AFIAS automated immunoassay system. HOMA-IR was calculated as fasting insulin (µU/mL) × fasting glucose (mg/dL) / 405 (Matthews *et al*., 1985).

2.3. Outcomes and Statistical Analysis

The primary biochemical outcomes were serum humanin and MOTS-c concentrations. Secondary outcomes were glycated hemoglobin, fasting glucose, insulin, and HOMA-IR. Quantitative variables were analyzed as continuous measures. Group differences were evaluated using one-way analysis of variance followed by the least significant difference post hoc test. Normality and homogeneity of variance were not formally assessed using the Shapiro-Wilk and Levene tests because archived participant-level diagnostic outputs were unavailable. Results are presented as mean ± standard error together with 95% confidence intervals.

Omnibus effects are reported as F(3, 84), P values, and eta-squared (η^2). All analyses were unadjusted because complete participant-level covariate data were not available for multivariable modeling; no subgroup or sensitivity analyses were performed. Statistical significance was defined as two-sided $P < 0.05$.

3. RESULTS AND DISCUSSION:

3.1. Results

Serum humanin concentrations did not differ significantly among the control [3.66 ± 0.55 pg/mL; 95% confidence interval (CI), 2.52–4.80], type 2 diabetes [2.88 ± 0.66 pg/mL; 95% CI, 1.51–4.25], atherosclerosis [2.83 ± 0.14 pg/mL; 95% CI, 2.54–3.12], and hypertension groups [2.66 ± 0.12 pg/mL; 95% CI, 2.41–2.91; $F(3, 84) = 1.013$, $P = 0.391$, $\eta^2 = 0.035$] (Figure 1; Table 1).

Serum MOTS-c concentrations were lower in the type 2 diabetes [17.77 ± 1.53 pg/mL; 95% CI, 14.59–20.95], atherosclerosis [19.78 ± 3.07 pg/mL; 95% CI, 13.40–26.16], and hypertension groups [18.27 ± 2.29 pg/mL; 95% CI, 13.51–23.03] than in the control group [99.27 ± 6.36 pg/mL; 95% CI, 86.04–112.50; $F(3, 84) = 113.339$, $P < 0.001$, $\eta^2 = 0.802$]. The three patient groups did not differ significantly from one another (Figure 2; Table 1).

Glycated hemoglobin was highest in the type 2 diabetes group [9.21 ± 0.46%; 95% CI, 8.25–10.17] and exceeded the values in the atherosclerosis [5.58 ± 0.07%; 95% CI, 5.43–5.73], hypertension [5.55 ± 0.06%; 95% CI, 5.43–5.67], and control groups [5.48 ± 0.07%; 95% CI, 5.33–5.63; $F(3, 84) = 58.676$, $P < 0.001$, $\eta^2 = 0.677$]. No significant differences were observed among the latter three groups (Figure 3; Table 1).

Fasting glucose differed significantly among groups [$F(3, 84) = 25.989$, $P < 0.001$, $\eta^2 = 0.481$]. The type 2 diabetes group had the highest concentration [210.09 ± 16.92 mg/dL; 95% CI, 174.90–245.28], followed by the hypertension [134.23 ± 14.54 mg/dL; 95% CI, 103.99–164.47], atherosclerosis [94.23 ± 2.72 mg/dL; 95% CI, 88.57–99.89], and control groups [81.50 ± 3.36 mg/dL; 95% CI, 74.51–88.49]. Post hoc comparisons are summarized by the letter annotations in Figure 4.

Serum insulin concentrations differed significantly among groups [$F(3, 84) = 4.965$, $P = 0.003$, $\eta^2 = 0.151$]. Mean concentrations were 9.24 ± 0.97 µU/mL (95% CI, 7.22–11.26) in controls, 23.00 ± 4.26 µU/mL (95% CI, 14.14–31.86) in type 2 diabetes, 17.55 ± 2.12 µU/mL (95% CI, 13.14–21.96) in atherosclerosis, and 19.78 ± 2.06 µU/mL (95% CI, 15.50–24.06) in hypertension. The post hoc pattern is shown in Figure 5.

HOMA-IR differed significantly among groups [$F(3, 84) = 10.203$, $P < 0.001$, $\eta^2 = 0.267$]. Values were highest in type 2 diabetes [8.05 ± 1.31; 95% CI, 5.33–10.77], followed by hypertension [5.40 ± 0.70; 95% CI, 3.94–6.86], atherosclerosis [4.16 ± 0.58; 95% CI, 2.95–5.37], and controls [1.86 ± 0.19; 95% CI, 1.46–2.26]. The pairwise comparison pattern is presented in Figure 6.

3.2. Discussion

The principal findings were a marked reduction in circulating MOTS-c across the three patient groups, no statistically significant group difference in humanin, and the greatest glycemic dysregulation and insulin resistance in men with type 2 diabetes. Because this was a cross-sectional, unadjusted comparison, these findings indicate associations rather than causal or predictive relationships.

The absence of a significant difference in humanin is consistent with the report by Conte *et al*. (2021), but contrasts with studies that observed lower circulating humanin in impaired fasting glucose or diabetes (Ramanjaneya *et al*., 2019; Voigt and Jelinek, 2016). Exercise status, age, disease severity, assay characteristics, and tissue-specific activity may contribute to this heterogeneity. Humanin concentrations can vary with age and growth hormone/insulin-like growth factor-1 signaling, and circulating levels may not fully represent biological activity within target tissues (Gong *et al*., 2014; Lee *et al*., 2014; Boutari *et al*., 2022). Evidence that resistance training can increase skeletal-muscle humanin levels in men with impaired glucose metabolism further supports exercise status as a potential source of between-study variation (Gidlund *et al*., 2016).

Humanin has also been linked to vascular and oxidative-stress phenotypes. Lower circulating humanin has been associated with coronary endothelial dysfunction (Widmer *et al*., 2013), whereas experimental ischemia/reperfusion studies have demonstrated changes in endogenous humanin

after injury (Thummasorn *et al*., 2016). Increased tissue expression has been reported in mitochondrial disorders characterized by substantial oxidative stress (Kariya *et al*., 2005). These context-dependent observations support cautious interpretation of a single serum measurement.

MOTS-c concentrations were substantially lower in men with type 2 diabetes, atherosclerosis, or hypertension than in controls. This pattern may reflect impaired mitochondrial stress signaling or reduced compensatory metabolic responses rather than a disease-specific effect. Reviews of diabetes-related evidence similarly describe reduced circulating MOTS-c in metabolic dysfunction, although stage of disease, obesity, and treatment may influence the measured concentration (Fang *et al*., 2025).

The type 2 diabetes finding agrees with Ramanjaneya *et al*. (2019), who reported lower circulating MOTS-c in diabetes, and with Ikonomidis *et al*. (2020), who linked low MOTS-c to adverse cardiovascular phenotypes in patients with type 2 diabetes and coronary artery disease. The much larger difference between controls and all patient groups in the present study produced a large omnibus effect size; nevertheless, replication with independent cohorts and assay validation is required.

In coronary artery disease, lower MOTS-c has been associated with lesion severity and complexity (Yaşar *et al*., 2022). Experimental studies also suggest that MOTS-c participates in mitochondrial dynamics, antioxidant defense, and exercise-related metabolic adaptation (Li *et al*., 2019; Tang *et al*., 2023; Yang *et al*., 2021; Woodhead and Merry, 2021). Reduced expression in chronic metabolic stress has also been described (Liu *et al*., 2019). These studies provide biologic plausibility, but they do not demonstrate that reduced MOTS-c caused the abnormalities observed in the present participants.

As expected, glycated hemoglobin, fasting glucose, and HOMA-IR were most markedly altered in the type 2 diabetes group. Recent evidence supports bidirectional crosstalk between impaired mitochondrial quality control and insulin resistance, whereby mitochondrial dysfunction may worsen insulin signaling, while hyperglycemia and other metabolic stressors can further compromise mitochondrial function (Chen *et al*., 2025). Nevertheless, the cross-sectional design of the present study precludes causal or directional inference. Accordingly, the lower MOTS-c concentrations observed alongside poorer glycemic indices should be interpreted as a group-level descriptive association rather than evidence of causality.

Previous experimental and clinical reports have linked mitochondrial-derived peptides to glucose homeostasis. Humanin-related peptides have altered metabolic profiles in obesity models (Mehta *et al*., 2019), and combined humanin, MOTS-c, and SHLP2 treatment improved glucose homeostasis in diabetic mice (Paul *et al*., 2026). Reviews and clinical studies have reported inverse associations of MOTS-c with fasting insulin, glycated hemoglobin, or insulin resistance, although findings are not uniform across comorbidity patterns (Miller *et al*., 2020; Ramanjaneya *et al*., 2019; Savvopoulos *et al*., 2024).

The higher fasting glucose observed in the hypertension group is compatible with epidemiologic evidence linking dysglycemia to later hypertension risk, particularly in men (Ahn *et al*., 2021). However, the current groups were not matched or adjusted for medication use, disease duration, adiposity, smoking, diet, or physical activity, and these factors could explain part of the between-group differences.

Insulin resistance is also relevant to atherosclerotic risk. In a prospective cohort of 929 adults, elevated insulin-resistance-related indices were associated with incident carotid plaques, including among individuals with comparatively favorable lipid or cardiovascular risk profiles (Liu *et al*., 2026). This supports the clinical importance of insulin resistance, but it does not directly validate HOMA-IR or MOTS-c as diagnostic markers in the present sample.

Clinically, MOTS-c may merit further study as a complementary biomarker of cardiometabolic dysfunction, but the substantial between-study variability, limited assay standardization, and overlap among disease mechanisms preclude immediate diagnostic use. Future work should include women, wider age ranges, larger multicenter samples, detailed medication and lifestyle data, validated assay performance, and longitudinal follow-up to determine temporal relationships and clinical utility.

4. CONCLUSIONS

In this exploratory cross-sectional study, circulating MOTS-c was markedly lower in men with type 2 diabetes mellitus, atherosclerosis, or hypertension than in healthy controls, whereas humanin did not differ significantly among groups. Glycated hemoglobin, fasting glucose, and HOMA-IR were most abnormal in type 2 diabetes. These findings support an association between reduced MOTS-c and cardiometabolic dysfunction but do not establish causality or diagnostic performance. Larger longitudinal and multicenter studies that include women, broader age groups, assay validation, and adjustment for medication and lifestyle factors are needed.

5. DECLARATIONS

5.1. Study Limitations

The sample comprised only men aged 40–60 years from a single geographical area, which limits external validity. The modest sample size (22 participants per group) was based on recruitment feasibility rather than a formal a priori power calculation and may have limited detection of smaller effects, particularly for humanin. Recruitment was site-based and non-random; blinding and randomization were not applicable to group assignment, and laboratory blinding was not documented. Medication use, disease duration, adiposity, smoking, diet, physical activity, and other potential confounders were not controlled, and analyses were not adjusted. Formal archived outputs for distributional assumptions and sensitivity analyses were unavailable. The cross-sectional design precludes causal inference, and circulating humanin may not reflect tissue-level activity. In addition, six biochemical outcomes were evaluated using LSD post hoc comparisons without a multiplicity correction, which may have increased the family-wise risk of type I error.

5.2. Acknowledgments:

The authors thank Dr. Assad Yahia for assistance with the statistical analysis and gratefully acknowledge all patients and healthy volunteers who participated in the study.

5.3. Funding Sources:

The authors funded this research.

5.4. Conflicts of Interest:

The authors declare that they have no financial, personal, or professional competing interests related to this work.

5.5. Data Availability:

Participant-level data are not publicly available because of ethical and regulatory requirements concerning privacy and confidentiality. De-identified aggregated data may be available from the corresponding author upon reasonable request and subject to institutional approval.

5.6. Author Contributions:

Inas Majid Mozan: CD, DC, DAI, MW, FA.

Zainab Abduljabber Ridha Al-Ali and Redha Alwan Hasan: CD, DAI, CR, FA.

Code	English	Português
CD	Conception and Design	Concepção e Design
DC	Data Collection	Coleta de Dados
DAI	Data Analysis and Interpretation	Análise e Interpretação de Dados
MW	Manuscript Writing	Escrita do Manuscrito
CR	Critical Review	Revisão Crítica
FA	Final Approval	Aprovação Final

5.7. AI and Computational Tools Declaration:

During manuscript preparation, the authors used ChatGPT (OpenAI, San Francisco, CA, USA) solely for language editing and readability. The tool was not used to generate study data, conduct laboratory procedures, perform the original statistical analysis, or determine scientific conclusions. All AI-assisted text was critically reviewed and edited by the authors, who verified the scientific content and accept full responsibility for the final manuscript.

5.8. Research Integrity Declaration:

The authors affirm that the study was conducted and reported according to accepted principles of accuracy, transparency, accountability, responsible data handling, and research integrity.

5.9. Originality and Similarity Statement:

The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere. The manuscript was screened for text similarity before submission.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN SUBJECTS

6.1. Ethics Committee Approval

The study protocol was approved by the Training and Human Development Center, Misan Health Directorate (approval no. 770, dated 27 November 2024). All participant-related procedures complied with applicable institutional ethical requirements and the principles of the Declaration of Helsinki.

6.2. Informed Consent

All participants received an explanation of the study procedures, sample collection, and laboratory investigations. Written and signed informed consent was obtained before enrollment and before any study-specific procedure.

7. REFERENCES:

1. Ahn, S. K., Lee, J. M., Ji, S. M., Kim, K. H., Park, J. H., and Hyun, M. K. (2021). Incidence of hypertension and fasting blood glucose from real-world data: retrospective cohort for 7-years follow-up. International Journal of Environmental Research and Public Health, 18(4), 2085. DOI:10.3390/ijerph18042085.
2. Boutari, C., Pappas, P. D., Theodoridis, T. D., and Vavilis, D. (2022). Humanin and diabetes mellitus: A review of in vitro and in vivo studies. World journal of diabetes, 13(3), 213. DOI: 10.4239/wjd.v13.i3.213
3. Chen, Y., Liu, X., Liu, Y., Li, Y., Li, D., Mei, Z., and Deng, Y. (2025). Mitochondrial quality control in diabetes mellitus and complications: molecular mechanisms and therapeutic strategies. *Cell Death and Disease*, *16*(1), 652. https://doi.org/10.1038/s41419-025-07936-y
4. Clemente-Suárez, V. J., Redondo-Flórez, L., Beltrán-Velasco, A. I., Ramos-Campo, D. J., Belinchón-deMiguel, P., Martinez-Guardado, I.,... and Tornero-Aguilera, J. F. (2023). Mitochondria and brain disease: a comprehensive review of pathological mechanisms and therapeutic opportunities. Biomedicines, 11(9), 2488. DOI: 10.3390/biomedicines11092488.
5. Conte, M., Sabbatinelli, J., Chiariello, A., Martucci, M., Santoro, A., Monti, D.,... and Salvioli, S. (2021). Disease-specific plasma levels of mitokines FGF21, GDF15, and Humanin in type II diabetes and Alzheimer's disease in comparison with healthy aging. Geroscience, 43(2), 985-1001.DOI:10.1007/s11357-020-00287-w. Epub 2020 Oct 31
6. Coradduzza, D., Congiargiu, A., Chen, Z., Cruciani, S., Zinellu, A., Carru, C., and Medici, S. (2023). Humanin and its pathophysiological roles in aging: A systematic review. Biology, 12(4), 558. DOI:10.3390/biology12040558.
7. Evangeline, S., and Iyer, P. R. (2021). Atherosclerosis: causes and cures. Healthc Rev, 2(1), 34-44. DOI:10.47285/hr.v2i1.87
8. Fang, T., Han, J. C., Taberner, A., and Pham, T. (2025). MOTS-c in type 2 diabetes mellitus: From risk factors to cardiac complications and potential treatment. Life Sciences, 124009. DOI: 10.1016/j.lfs.2025.124009. Epub 2025 Oct 11.
9. Gidlund, E. K., von Walden, F., Venojärvi, M., Risérus, U., Heinonen, O. J., Norrbom, J., and Sundberg, C. J. (2016). Human skeletal muscle protein levels increase after resistance training in men with impaired glucose metabolism. Physiological reports, 4(23), e13063. DOI:10.14814/phy2.13063...
10. Gong, Z., Tas, E., and Muzumdar, R. (2014). Humanin and age-related diseases: A new link? Frontiers in Endocrinology, 5, 210. https://doi.org/10.3389/fendo.2014.00210
11. Hashimoto, Y., Niikura, T., Ito, Y., Sudo, H., Hata, M., Arakawa, E., *et al*. (2001). Detailed characterization of neuroprotection by a rescue factor humanin against various

Alzheimer's disease-relevant insults. Journal of Neuroscience, 21(23), 9235–9245. https://doi.org/10.1523/JNEUROSCI.21-23-09235.2001
12. Hoang, P. T., Park, P., Cobb, L. J., Paharkova-Vatchkova, V., Hakimi, M., Cohen, P., and Lee, K. W. (2010). The neurosurvival factor Humanin inhibits β-cell apoptosis via signal transducer and activator of transcription 3 activation and delays and ameliorates diabetes in nonobese diabetic mice. Metabolism, 59(3),343-349. DOI:10.1016/j.metabol.2009.08.001. Epub 2009 Oct 1.
13. Ikonomidis, I., Katogiannis, K., Kyriakou, E., Taichert, M., Katsimaglis, G., Tsoumani, M., *et al*. (2020). β-Amyloid and mitochondrial-derived peptide-c are additive predictors of adverse outcome to high-on-treatment platelet reactivity in type 2 diabetics with revascularized coronary artery disease. Journal of Thrombosis and Thrombolysis, 49(3), 365–376. https://doi.org/10.1007/s11239-020-02060-4
14. Kariya, S., Hirano, M., Furiya, Y., Sugie, K., and Ueno, S. (2005). Humanin detected in skeletal muscles of MELAS patients: a possible new therapeutic agent. Acta neuropathologica, 109(4), 367-372. DOI: 10.1007/s00401-004-0965-5
15. Kim, S. J., Miller, B., Mehta, H. H., Xiao, J., Wan, J., Arpawong, T. E.,... and Cohen, P. (2019). The mitochondrial-derived peptide MOTS-c is a regulator of plasma metabolites and enhances insulin sensitivity. Physiological reports, 7(13), e14171. DOI:10.14814/phy2.14171.
16. Kumagai, H., Miller, B., Kim, S. J., Leelaprachakul, N., Kikuchi, N., Yen, K., and Cohen, P. (2023). Novel insights into mitochondrial DNA: Mitochondrial microproteins and mtDNA variants modulate athletic performance and age-related diseases. Genes, 14(2), 286. DOI:10.3390/genes14020286.
17. Lee, C., Wan, J., Miyazaki, B., Fang, Y., Guevara-Aguirre, J., Yen, K.,... and Cohen, P. (2014). IGF-I regulates the age-dependent signaling peptide humanin. Aging cell, 13(5), 958-961. DOI:10.1111/acel.12243
18. Lee, C., Zeng, J., Drew, B. G., Sallam, T., Martin-Montalvo, A., Wan, J.,... and Cohen, P. (2015). The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. Cell Metabolism, 21(3), 443-454.DOI:10.1016/j.cmet.2015.02.009.
19. Li, Q., Lu, H., Hu, G., Ye, Z., Zhai, D., Yan, Z.,... and Lu, Z. (2019). Earlier changes in mice after D-galactose treatment were improved by mitochondria-derived small peptide MOTS-c. Biochemical and Biophysical Research Communications, 513(2), 439-445. DOI:10.1016/j.bbrc.2019.03.194.
20. Liu, C., Gidlund, E. K., Witasp, A., Qureshi, A. R., Söderberg, M., Thorell, A.,... and von Walden, F. (2019). Reduced skeletal muscle expression of mitochondrial-derived peptides humanin and MOTS-C and Nrf2 in chronic kidney disease. American Journal of Physiology-Renal Physiology, 317(5), F1122-F1131. DOI:10.1152/ajprenal.00202.2019
21. Liu, P., Wang, H., Han, X., Jin, R., Li, J., Song, S., *et al*. (2026). Associations of insulin resistance-related indices with incident carotid plaques by cardiovascular risk profiles: A prospective cohort study. Lipids in Health and Disease, 25, 72. https://doi.org/10.1186/s12944-026-02881-x
22. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28(7), 412–419. https://doi.org/10.1007/BF00280883
23. Mehta, H. H., Xiao, J., Ramirez, R., Miller, B., Kim, S. J., Cohen, P., and Yen, K. (2019). Metabolomic profile of diet-induced obesity mice in response to humanin and small humanin-like peptide 2 treatment. Metabolomics, 15(6), 88. https://doi.org/10.1007/s11306-019-1549-7
24. Merry, T. L., Chan, A., Woodhead, J. S., Reynolds, J. C., Kumagai, H., Kim, S. J., and Lee, C. (2020). Mitochondrial-derived peptides in energy metabolism. American Journal of Physiology-Endocrinology and Metabolism, 319(4), E659-E666. DOI:10.1152/ajpendo.00249.2020. Epub 2020 Aug 10.
25. Miller, B., Kim, S. J., Kumagai, H., Mehta, H. H., Xiang, W., Liu, J.,... and Cohen, P. (2020). Peptides derived from small mitochondrial open reading frames: Genomic, biological, and therapeutic implications. Experimental Cell Research, 393(2), 112056. DOI:10.1016/j.yexcr.2020.112056.
26. Nathan, D. M., Kuenen, J., Borg, R., Zheng,

H., Schoenfeld, D., Heine, R. J., and A1c-Derived Average Glucose Study Group. (2008). Translating the A1C assay into estimated average glucose values. Diabetes Care, 31(8), 1473–1478. https://doi.org/10.2337/dc08-0545

27. Paul, T., Banerjee, O., Das, S., Maji, B. K., Goswami, S., and Mukherjee, S. (2026). Mitochondrial-derived peptides (humanin, MOTS-c, and SHLP2) protect pancreatic islet cells and delay the onset and progression of type 2 diabetes mellitus in mice. PharmaNutrition, 35, 100480. https://doi.org/10.1016/j.phanu.2026.100480
28. Ramanjaneya, M., Bettahi, I., Jerobin, J., Chandra, P., Abi Khalil, C., Skarulis, M., *et al*. (2019). Mitochondrial-derived peptides are downregulated in diabetes subjects. Frontiers in Endocrinology, 10, 331. https://doi.org/10.3389/fendo.2019.00331
29. Saghatelian, A., and Couso, J. P. (2015). Discovery and characterization of smORF-encoded bioactive polypeptides. Nature Chemical Biology, 11(12), 909-916. DOI:10.1038/nchembio.1964.
30. Salemi, M., Ridolfo, F., Salluzzo, M. G., Cannarrella, R., Giambirtone, M., Caniglia, S.,... and Romano, C. (2020). Humanin gene expression in fibroblasts of Down syndrome subjects. International Journal of Medical Sciences, 17(3), 320. DOI:10.7150/ijms.39145.eCollection 2020
31. Saminathan, A., Zajac, M., Anees, P., and Krishnan, Y. (2022). Organelle-level precision with next-generation targeting technologies. Nature Reviews Materials, 7(5), 355-371. DOI:10.1038/s41578-021-00396-8
32. Savvopoulos, S., Hatzikirou, H., and Jelinek, H. F. (2024). Comparative analysis of biomarkers in type 2 diabetes patients with and without comorbidities: insights into the role of hypertension and cardiovascular disease. Biomarker Insights, 19, 11772719231222111. DOI:10.1177/11772719231222111
33. Schleicher, E., Gerdes, C., Petersmann, A., Müller-Wieland, D., Müller, U. A., Freckmann, G.,... and Landgraf, R. (2022). Definition, classification and diagnosis of diabetes mellitus. Experimental and Clinical Endocrinology and Diabetes, 130(S 01), S1-S8. DOI: 10.1055/a-1624-2897 .
34. Tang, M., Su, Q., Duan, Y., Fu, Y., Liang, M., Pan, Y.,... and Li, S. (2023). The role of MOTS-c-mediated antioxidant defense in aerobic exercise alleviating diabetic myocardial injury. Scientific Reports, 13(1), 19781. DOI:10.1038/s41598-023-47073-0.
35. Thummasorn, S., Apaijai, N., Kerdphoo, S., Shinlapawittayatorn, K., Chattipakorn, S. C., and Chattipakorn, N. (2016). Humanin exerts cardioprotection against cardiac ischemia/reperfusion injury through attenuation of mitochondrial dysfunction. Cardiovascular therapeutics, 34(6), 404-414. DOI: 10.1111/1755-5922.12210
36. Voigt, A., and Jelinek, H. F. (2016). Humanin: a mitochondrial signaling peptide as a biomarker for impaired fasting glucose-related oxidative stress. Physiological reports, 4(9), e12796. DOI: 10.14814/phy2.12796
37. Widmer, R. J., Flammer, A. J., Herrmann, J., Rodriguez-Porcel, M., Wan, J., Cohen, P.,... and Lerman, A. (2013). Circulating humanin levels are associated with preserved coronary endothelial function. American Journal of Physiology-Heart and Circulatory Physiology, 304(3), H393-H397. DOI: 10.1152/ajpheart.00765.2012.
38. Woodhead, J. S., and Merry, T. L. (2021). Mitochondrial-derived peptides and exercise. Biochimica et Biophysica Acta (BBA)-General Subjects, 1865(12), 130011. DOI: 10.1016/j.bbagen.2021.130011
39. Yang, B., Yu, Q., Chang, B., Guo, Q., Xu, S., Yi, X., and Cao, S. (2021). MOTS-c interacts synergistically with exercise intervention to regulate PGC-1α expression, attenuate insulin resistance and enhance glucose metabolism in mice via AMPK signaling pathway. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1867(6), 166126. DOI:10.1016/j.bbadis.2021.166126.
40. Yaşar, E., Çakmak, T., Bayramoğlu, A., Karakuş, Y., Tekin, S., Şekerci, G., and Türkoğlu, C. (2022). MOTS-c as a predictor of coronary lesions and complexity in patients with stable coronary artery disease. European Review for Medical and Pharmacological Sciences, 26(16). DOI:10.26355/eurrev_202208_29501.
41. Zhu, W. W., Wang, S. R., Liu, Z. H., Cao, Y. J., Wang, F., Wang, J.,... and Zhang, Y. L. (2017). Gly [14]-humanin inhibits ox-LDL uptake and stimulates cholesterol efflux in macrophage-derived foam cells. Biochemical and Biophysical Research Communications, 482(1), 93-99. DOI: 10.1016/j.bbrc.2016.10.138
42. Zuccato, C. F., Asad, A. S., Nicola Candia,

A. J., Gottardo, M. F., Moreno Ayala, M. A., Theas, M. S.,... and Candolfi, M. (2019). Mitochondrial-derived peptide humanin as therapeutic target in cancer and degenerative diseases. Expert opinion on therapeutic targets, 23(2), 117-126.DOI:10.1080/14728222.2019.1559300.

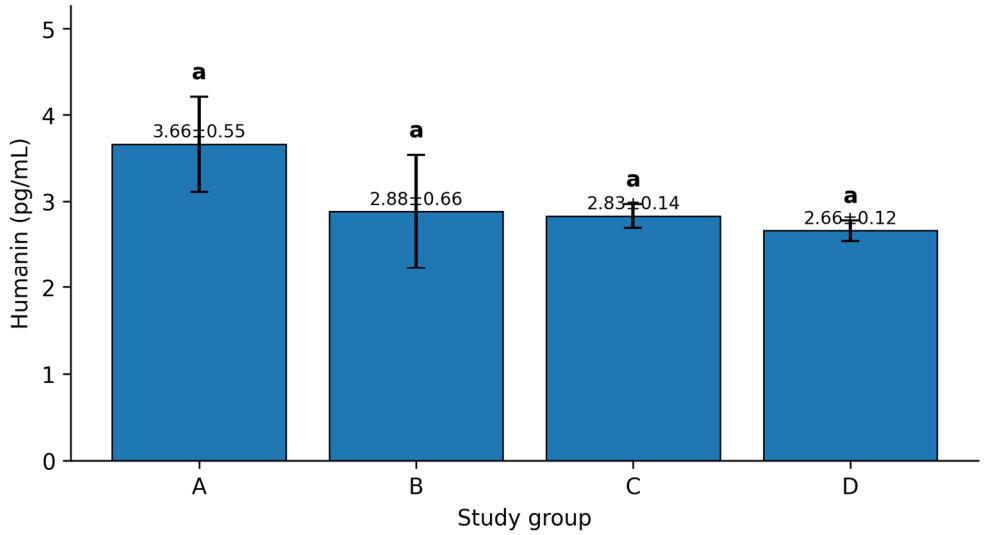

***Figure 1**. Serum humanin concentrations by study group. A, healthy control; B, type 2 diabetes mellitus; C, atherosclerosis; D, hypertension. Bars represent mean ± standard error. Identical letters indicate no significant difference according to the least significant difference post hoc test (P > 0.05).*

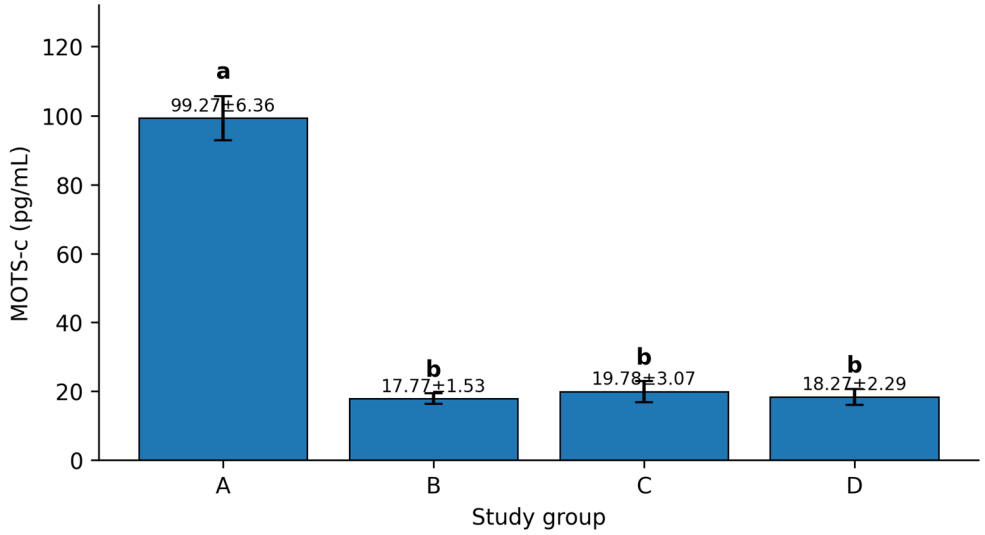

***Figure 2**. Serum MOTS-c concentrations by study group. A, healthy control; B, type 2 diabetes mellitus; C, atherosclerosis; D, hypertension. Bars represent mean ± standard error. Different letters indicate significant pairwise differences, whereas identical letters indicate no significant difference (P < 0.05).*

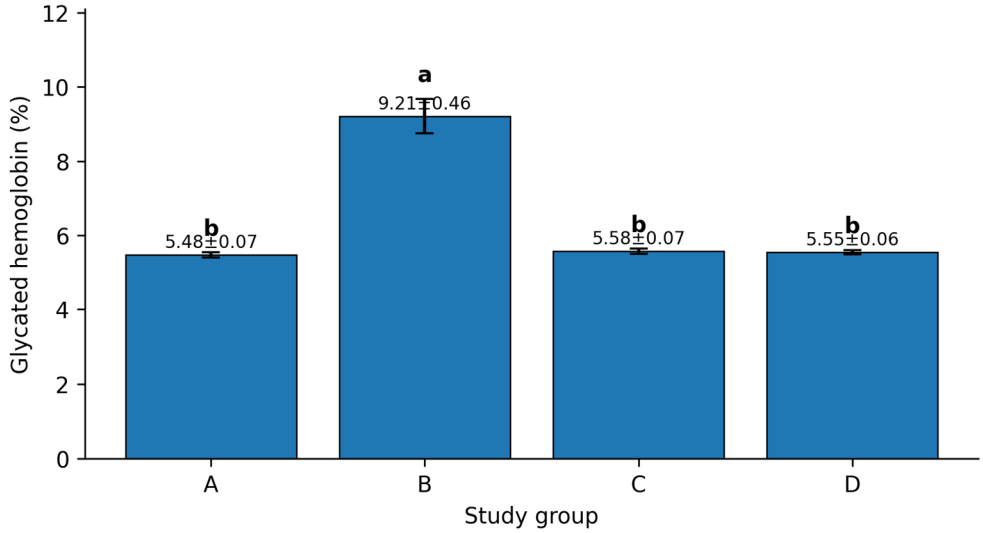

Figure 3. *Glycated hemoglobin by study group. A, healthy control; B, type 2 diabetes mellitus; C, atherosclerosis; D, hypertension. Bars represent mean ± standard error. Different letters indicate significant pairwise differences (P < 0.05).*

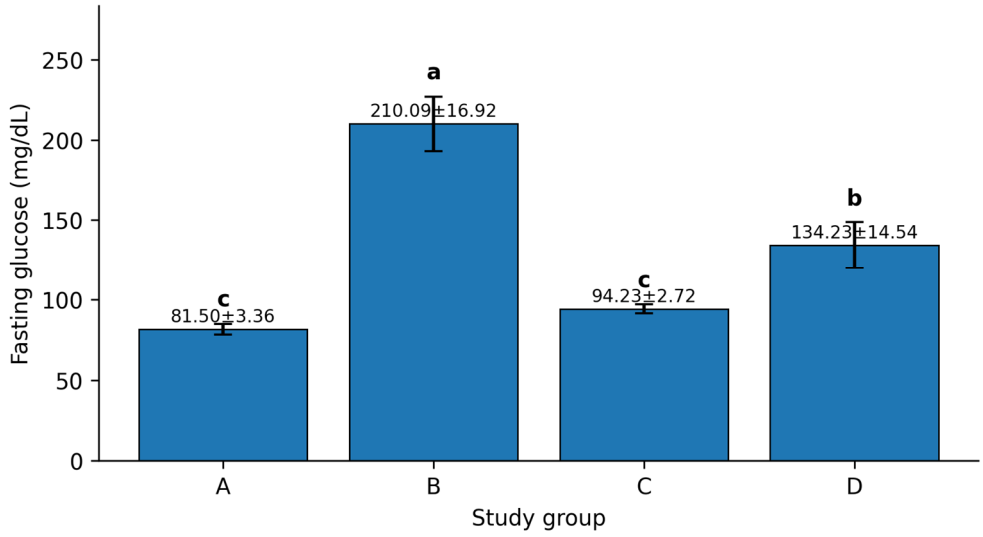

Figure 4. *Fasting blood glucose by study group. A, healthy control; B, type 2 diabetes mellitus; C, atherosclerosis; D, hypertension. Bars represent mean ± standard error. Letter annotations summarize least significant difference post hoc comparisons (P < 0.05).*

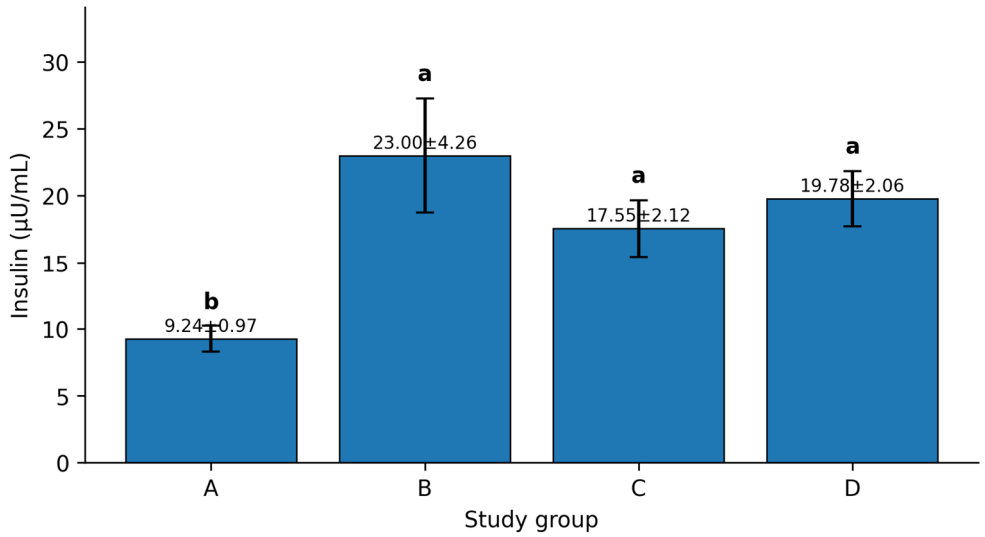

Figure 5. *Serum insulin concentrations by study group. A, healthy control; B, type 2 diabetes mellitus; C, atherosclerosis; D, hypertension. Bars represent mean ± standard error. Letter annotations summarize least significant difference post hoc comparisons (P < 0.05).*

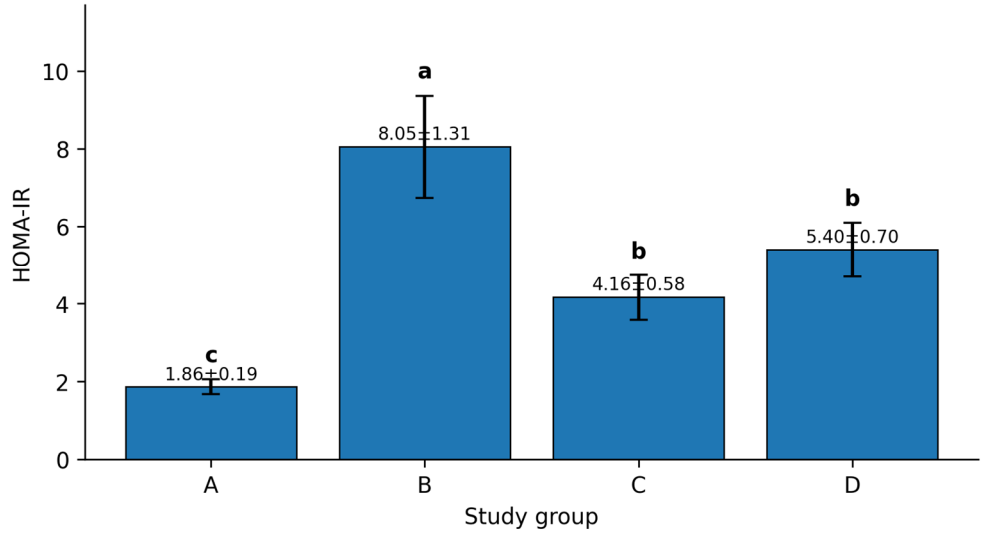

Figure 6*. Homeostatic model assessment of insulin resistance (HOMA-IR) by study group. A, healthy control; B, type 2 diabetes mellitus; C, atherosclerosis; D, hypertension. Bars represent mean ± standard error. Letter annotations summarize least significant difference post hoc comparisons (P < 0.05).*

Table 1. *One-way analysis of variance results for the six biochemical outcomes.*

Parameter	ANOVA df	F	P value	η²
Humanin	3, 84	1.013	0.391	0.035
MOTS-c	3, 84	113.339	<0.001	0.802
HbA1c	3, 84	58.676	<0.001	0.677
Glucose	3, 84	25.989	<0.001	0.481
Insulin	3, 84	4.965	0.003	0.151
HOMA-IR	3, 84	10.203	<0.001	0.267

For each outcome, df = 3, 84. Values are based on one-way analysis of variance; η² denotes eta-squared effect size. P values below 0.001 are reported as P < 0.001. Pairwise comparisons were performed using the least significant difference post hoc test.