

Biochemical Study of Isthmin-1 in Obese Males of Mosul City-Iraq

^{1*}Abeer A. Hazaa, ²Safaa A. Al-Ameen

^{1,2}College of Science, Department of Chemistry, University of Mosul, Mosul, Iraq

Authors E-mail: abeer.24scp7@student.uomosul.edu.iq, safaalameen@uomosul.edu.iq

Abstract - Obesity represents a significant global health issue in the 21st century, as it is linked to a higher risk of numerous diseases. It significantly affects several body functions, including immune response, as well as the endocrine and paracrine functions of adipose tissue. Isthmin-1 is a newly identified adipokine with insulin-like properties, secreted by adipose tissue. It stimulates a series of signaling pathways like those triggered by insulin, enhancing glucose uptake, inhibiting lipid synthesis in the liver and promoting protein synthesis. In this study, 5 ml of blood samples were collected from 65 obese men aged between 18 and 60 years from hospitals in Mosul. Participants were categorized based on their body mass index (BMI). The findings revealed a significant elevation ($P \leq 0.05$) in isthmin-1 levels among obese individuals compared to the control group, with a positive correlation identified between isthmin-1 levels and BMI. Additionally, elevated levels of triglycerides and cholesterol were recorded in the obese group. The approximate molecular weight of isthmin-1 was also determined using biochemical techniques, it was found to be around 64 kilo daltons, with a specific activity of 7.48, a purification fold of 23.3, and a recovery rate of 77%.

Keywords: Obesity, Isthmin-1, Gel filtration, cholesterol, triglycerides.

I. INTRODUCTION

Obesity is increasingly recognized as one of the most critical global health challenges of the 21st century [1]. The World Health Organization (WHO) classifies obesity as a disease due to its strong association with an elevated risk of metabolic disorders, including insulin resistance and type 2 diabetes [2,3]. This condition typically arises from a persistent imbalance between energy intake and expenditure; whereby excessive caloric consumption surpasses energy utilization. As a result, surplus energy is stored predominantly in adipose tissue as fat. Beyond its role as the primary energy reservoir through triglyceride storage, adipose tissue also acts as an endocrine organ, secreting various bioactive molecules namely adipokines and cytokines that regulate metabolic functions,

maintain energy homeostasis, influence insulin sensitivity, and modulate immune and inflammatory responses [4,5].

Recent studies by Jiang Z and colleagues. have identified Isthmin-1 (ISM-1) as a novel adipokine secreted by adipose tissue. ISM-1 exhibits insulin-like activity by activating similar intracellular signaling pathways. It promotes glucose uptake via an insulin-independent mechanism, suppresses hepatic lipogenesis, and enhances protein biosynthesis [6,7]. These characteristics suggest that ISM-1 may serve as a potential therapeutic target for the treatment of metabolic disorders such as type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) [8].

ISM-1 has attracted increasing attention due to its insulin-mimetic properties and its association with obesity. Research has demonstrated a positive correlation between circulating ISM-1 levels and subcutaneous fat mass, as well as with the subcutaneous fat area to visceral fat area (SFA/VFA) ratio. Notably, no significant association has been observed between ISM-1 levels and visceral fat, indicating that subcutaneous adipocytes may be the principal source of ISM-1 secretion. These findings support the hypothesis that ISM-1 may exert endocrine functions contributing to systemic energy regulation [9]. Furthermore, elevated serum ISM-1 levels have been reported in obese adolescent males, while no corresponding increase was noted in females of the same age group. ISM-1 levels have also shown a positive correlation with body mass index (BMI) and total body fat mass [10].

II. MATERIALS AND METHODS

This study involved the collection of blood samples from various hospitals in Mosul. A total of 65 obese males aged between 18 and 60 years were enrolled and categorized into four groups based on their body mass index (BMI). An additional 30 healthy males with normal BMI served as the control group. All blood samples were obtained in a fasting state. The blood was collected into gel-containing tubes and incubated in a water bath at 37°C for 10 minutes. The serum was separated via centrifugation at 4000 rpm for 10 minutes and then stored in clean, dry plastic tubes at -20°C, after being divided into aliquots for subsequent biochemical analyses. Informed consent was obtained from all participants prior to

blood sample collection. Obese individuals included in the study were free from any chronic diseases. Participants with a history of hypertension, thyroid disorders, diabetes mellitus, kidney diseases, or liver diseases were excluded from the study.

Additionally, demographic and health-related data were collected from all participants, including age, weight, height, family medical history, dietary habits, physical activity status, smoking status, occupation, and marital status.

The serum concentration of ISM-1 was measured using an ELISA kit provided by BT-LAB, China, based on the enzyme-linked immunosorbent assay (ELISA) technique as shown in Table 1.

Cholesterol and Triglycerides were estimated by using Cormay kit in an Accent 200 device, as shown in Table 2.

Isolation and partial purification of Isthmin-1 from normal human plasma:

This section outlines the isolation and partial purification of Isthmin-1 from the plasma of a healthy 28-year-old male donor. The procedure utilized a series of biotechnological methods, detailed as follows:

Protein Precipitation and Separation:

Plasma proteins were precipitated using 70% saturated ammonium sulfate at 4°C with gentle stirring for 60 minutes. Ammonium sulfate acts by binding to water molecules, reducing the amount of free water available to proteins, thus encouraging protein aggregation and precipitation. The mixture was then refrigerated for 24 hours to ensure complete protein precipitation [11].

Following the precipitation, cold centrifugation was performed at 10,000 rpm for 50 minutes to separate the precipitate from the supernatant. The resulting protein pellet was transferred into a clean, dry tube and stored at -20°C for further processing [12].

Salt and Small Molecule Removal (Dialysis):

To remove salt and low-molecular-weight substances, dialysis was conducted using cellophane tubing, a semi-permeable membrane that allows small molecules to pass through while retaining larger ones. This procedure was conducted at 4°C with constant gentle stirring via a magnetic stirrer over a 24-hour period, during which the 0.1 M ammonium bicarbonate solution was regularly refreshed. Following dialysis, the solution was concentrated using a lyophilizer.

Gel Filtration Chromatography:

A chromatography column measuring 75 × 1.5 cm was packed with Sephadex G-100 to a final bed height of 65 cm, serving as the stationary phase. The protein sample, along with molecular weight standards ranging from 204 to 2,000,000 Daltons, was applied to the column to determine essential parameters, including the internal volume (Vi) and void volume (Vo). This setup also enabled the isolation of Isthmin-1 and the estimation of its molecular weight. Elution was performed at a flow rate of 2 mL every 2 minutes. Protein elution was monitored by measuring absorbance at 280 nm using a UV-Vis spectrophotometer (Zenith Lab Co., China), the protein fractions obtained from gel filtration were freeze-dried (lyophilized) and stored at -20°C for further analysis.

Protein Concentration Estimation: Protein concentration was quantified using the modified Lowry method [13]. Bovine Serum Albumin (BSA) served as the standard protein. Absorbance was measured at 650 nm, and BSA, with a specific extinction coefficient of 0.67 mL mg⁻¹ cm⁻¹, was used to generate a standard curve. The absorbance values were directly proportional to protein concentrations [14,15].

III. RESULT AND DISCUSSION

Part One: Clinical study

Table 1: Isthmin-1 concentration (Mean ± S.E) in obese individuals compared to the control group, categorized by body mass index

BMI(Kg/m ²)	Isthmin-1 ng/ml Mean ±S. E
Normal (18.5-24.99) Control	6.24 ± 0.29
Overweight (25-29.99)	6.82 ± 0.33
Obese Class 1 (30-34.99)	7.41 ± 0.66
Obese Class 2 (35-39.99)	7.94 ± 0.58
Obese Class 3(BMI≥40)	8.55 ± 0.68

Significant at P ≤ 0.05

Table (1) indicates that serum levels of Isthmin-1 (ISM1) are significantly elevated in obese patients compared to the control group (P ≤ 0.05). Additionally, a positive correlation was observed between ISM1 levels and body mass index (BMI) (r = +0.284, P ≤ 0.01). Ruiz and colleagues. reported that increased ISM1 concentrations are linked to greater body fat mass [7]. Similarly, Yus and colleagues. proposed that the elevation in ISM1 levels may be due to increased secretion from subcutaneous adipocytes, suggesting a possible endocrine function of ISM1 in influencing other tissues [8].

Table 2: Cholesterol and triglyceride levels (Mean ± S.E) in obese patients versus the control group, based on body mass index classifications

BMI (Kg/m ²)	Cholesterol mg/dl Mean ± S. E	Triglyceride mg/dl Mean ± S. E
Normal (18.5-24.99) Control	128.21 ± 4.74	91 ± 4.31
Overweight (25-29.99)	163.34 ± 7.6	130.47 ± 10.23
Obese Class 1 (30-34.99)	176.13 ± 7.46	140 ± 18.64
Obese Class 2 (35-39.99)	183.6 ± 8.98	194.04 ± 23.54
Obese Class 3 (BMI ≥ 40)	216.08 ± 13.96	241.8 ± 34.069

Significant at P ≤ 0.01

Table (2) shows a significant increase in cholesterol and triglyceride levels in obese patients compared to the control group (P ≤ 0.01). Additionally, a positive correlation was identified between these lipid markers and body mass index (P ≤ 0.01). This rise is linked to obesity-related disruptions in lipid metabolism, which involve greater accumulation of lipid markers in adipose tissue and an increased release of free fatty acids into the bloodstream. These fatty acids are then used by the liver for triglyceride synthesis, leading to higher levels of these lipids in circulation [16,17].

Part Two: Isolation and Partial purification of Isthmin-1

-Gel Filtration Chromatography

Gel-filtration chromatography is a form of partition chromatography that separates molecules based on differences in their molecular size [18], as shown in Figure 1.

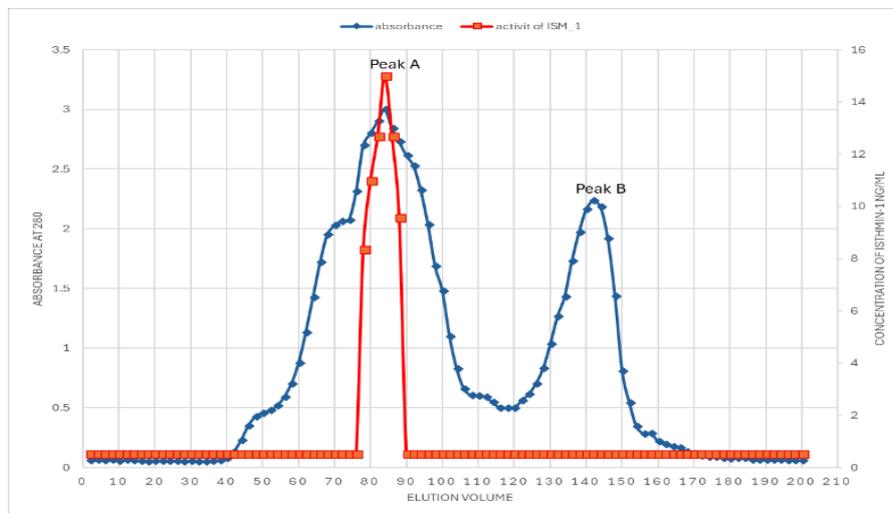


Figure 1: Elution volume of partial purified Isthmin-1 from normal human plasma by ammonium sulfate (70%) by gel filtration on the column (1.5 x 75) cm Sephadex G-100

The protein precipitate was separated into two peaks, A and B, based on molecular weight. Peak A showed high activity of Isthmin-1 and had a higher molecular weight, as shown in Figure 1. The total protein amount, Isthmin-1 concentration, specific activity, and purification folds are presented in Table 3.

Table 3: Step of partial purification of Isthmin-1 from normal plasma by gel filtration

Purification step	Total Volume (ml)	Total Protein (mg)	Total Conc. of Isthmin-1 (ng/ml)	Specific activity	No. of purification	Recovery %
Plasma	40	1060	349	0.32	1	100
Proteinous precipitate	20	898	313	0.34	1.062	89

Dialysis	30	604	286.8	0.47	1.46	82
Gel filtration/Sephadex G-100 (peak A) after lyophilized	26	36.3	271.6	7.48	23.3	77

The known molecular weights of the standard materials and the approximate molecular weight of Isthmin-1 are shown in Figure 2. The approximate molecular weight was found to be around 64 kDa, with a specific activity of (7.48), the recovery rate of (77%), and purification folds of (23.3), as determined using biochemical techniques. These results are consistent with the findings of Jang and his colleagues, who reported that the apparent molecular weight of Isthmin-1 is approximately 60 kDa [7,19,20,21].

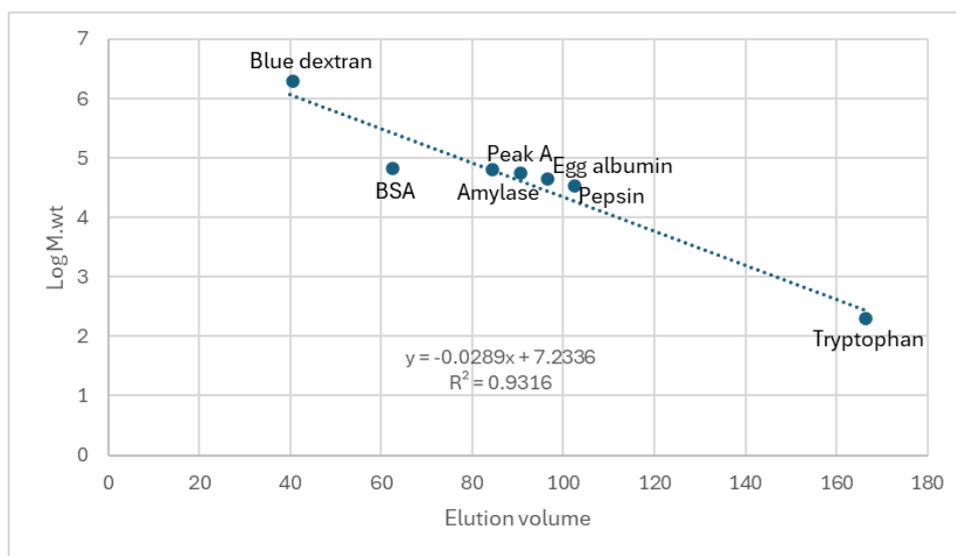


Figure 2: A graph showing the relationship between the logarithm of the molecular weights of standard proteins and their elution volumes on a Sephadex G-100 column (1.5 × 75 cm)

IV. CONCLUSION

Obesity emerged as a major global health concern in the 20th century, strongly linked to an increased risk of numerous chronic conditions. Isthmin-1, a newly identified adipokine with insulin-mimetic properties, has shown a strong association with obesity. Studies have revealed that ISM1 levels are elevated in obese individuals and are positively correlated with body mass index (BMI). Moreover, evidence indicates that obesity contributes to elevated levels of blood cholesterol and triglycerides, thereby amplifying the risk of related metabolic complications.

REFERENCES

- [1] World Health Organization, "Obesity and overweight," 9 June 2021. [Online]. Available: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>
- [2] K. A. Erion and B. E. Corkey, "Hyperinsulinemia: a cause of obesity?" *Curr. Obes. Rep.*, vol. 6, pp. 178–186, 2017.
- [3] J. Cawley and C. Meyerhoefer, "The medical care costs of obesity: an instrumental variables approach," *J. Health Econ.*, vol. 31, no. 1, pp. 219–230, 2012.
- [4] L. Liu et al., "Adipokines, adiposity, and atherosclerosis," *Cell. Mol. Life Sci.*, vol. 79, no. 5, p. 272, 2022.
- [5] T. Farkhondeh et al., "An overview of the role of adipokines in cardiometabolic diseases," *Molecules*, vol. 25, no. 21, p. 5218, 2020.
- [6] C. Li, S. Zhong, S. Ni, Z. Liu, S. Zhang, and G. Ji, "Zebrafish Ism1 is a novel antiviral factor that positively regulates antiviral immune responses," *Dev. Comp. Immunol.*, vol. 125, p. 104210, 2021, doi: 10.1016/j.dci.2021.104210.
- [7] Z. Jiang et al., "Isthmin-1 is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis," *Cell Metab.*, vol. 33, pp. 1836–1852.e11, 2021, doi: 10.1016/j.cmet.2021.07.010.
- [8] J. Heeren and L. Scheja, "Isthmin 1 – a novel insulin-like adipokine," *Nat. Rev. Endocrinol.*, vol. 17, pp. 709–710, 2021.
- [9] M. Lopez-Yus et al., "Isthmin-1 (ISM1), a novel adipokine that reflects abdominal adipose tissue

- distribution in individuals with obesity,” *Cardiovasc. Diabetol.*, vol. 22, no. 1, p. 335, 2023, doi: 10.1186/s12933-023-02075-0.
- [10] F. J. Ruiz-Ojeda et al., “Serum levels of the novel adipokine isthmin-1 are associated with obesity in pubertal boys,” *World J. Pediatr.*, vol. 19, no. 3, pp. 256–266, 2023, doi: 10.1007/s12519-022-00665-8.
- [11] F. J. Robyt and J. B. White, *Biochemical Techniques: Theory and Practice, U.S.A.: Cole Publishing Co., 1987*, pp. 141, 235–236, 246, 263, 296.
- [12] V. W. Rodwell, D. A. Bender, K. M. Botham, P. J. Kennelly, and P. A. Weil, *Harper's Illustrated Biochemistry, 30th ed., McGraw-Hill Education, 2018*.
- [13] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, “Protein measurement with the Folin phenol reagent,” *J. Biol. Chem.*, vol. 193, no. 1, pp. 265–275, 1951.
- [14] D. J. Holme and H. Peck, *Analytical Biochemistry, New York: John Wiley & Sons, 1988*, p. 86.
- [15] G. R. Schacterle and S. Pollack, “A simplified method for the quantitative assay of small amounts of protein in biological materials,” *Anal. Biochem.*, vol. 51, pp. 654–655, 1973.
- [16] P. A. Felig and K. R. Feingold, “Obesity and dyslipidemia,” in *Endotext*, K. R. Feingold, Ed., *National Center for Biotechnology Information (NCBI), 2023*. [Online]. Available: <https://www.endotext.org>.
- [17] H. E. Bays et al., “Obesity, dyslipidemia, and cardiovascular disease: A joint expert review from the Obesity Medicine Association and the National Lipid Association,” *Obes. Pillars*, vol. 10, p. 100108, 2024, doi: 10.1016/j.obpill.2024.100108.
- [18] C. Ó'Fágáin, P. M. Cummins, and B. F. O'Connor, “Gel-filtration chromatography,” *Methods Mol. Biol.*, vol. 1485, pp. 15–25, 2017, doi: 10.1007/978-1-4939-6412-3_2.
- [19] L. Menghuan et al., “Advances in research of biological functions of Isthmin-1,” *J. Cell Commun. Signal.*, 2023, doi: 10.1007/s12079-023-00732-3.
- [20] T. Li et al., “Crystal structure of Isthmin-1 and reassessment of its functional role in pre-adipocyte signaling,” *Nat. Commun.*, vol. 16, p. 3580, 2025, doi: 10.1038/s41467-025-58828-w.
- [21] T. Y. Ahmad, F. K. H. Tawfeeq, and S. A. Al-Ameen, “Biochemical study of dipeptidylpeptidase-4 from normal human serum,” *Rafidain J. Sci.*, vol. 27, no. 1, pp. 123–132, 2018.

Citation of this Article:

Abeer A. Hazaa, & Safaa A. Al-Ameen. (2025). Biochemical Study of Isthmin-1 in Obese Males of Mosul City-Iraq. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 9(6), 1-5. Article DOI <https://doi.org/10.47001/IRJIET/2025.906001>
