

تعيين مستويات الإنزيم المقوض للأنسولين المصلية لدى مرضى سوريين مصابين بالمتلازمة الاستقلابية بدون السكري من النمط الثاني

Determination of Serum Insulin Degrading Enzyme Levels in Syrian Patients with Metabolic Syndrome without Type 2 Diabetes Mellitus

صفاء عبد الهادي حوى^{1*}، يوسف أحمد بركات¹، زينب حيدر العرفي²

Safaa Abdulhadi Hawa^{1*}, Youssef Ahmad Barakat¹, and Zaynab Haydar Alourfi²

¹ قسم الطب المخبري، كلية الطب البشري، جامعة دمشق، دمشق، سورية

² قسم الأمراض الباطنة، كلية الطب البشري، جامعة دمشق، دمشق، سورية

¹ Department of Laboratory Medicine, Faculty of Medicine, Damascus University, Damascus, Syria

² Department of Internal Medicine, Faculty of Medicine, Damascus University, Damascus, Syria

المخلص Abstract:

خلفية البحث وهدفه: تشكل المتلازمة الاستقلابية MetS مجموعة من الاضطرابات الاستقلابية التي تتضمن ارتفاع غلوكوز الدم و/أو مقاومة الأنسولين واضطراب شحيمات الدم والبدانة المركزية وارتفاع ضغط الدم. وهي سبب هام للمراضة والوفيات، ويُعتقد أن سببها يتضمن تفاعلاً معقداً بين الاستعداد الوراثي والعوامل البيئية، وأهمها مقاومة الأنسولين. ومن ناحية أخرى، الإنزيم المقوض للأنسولين IDE هو الإنزيم الرئيس الذي يقوض الأنسولين، وتشير الدراسات الحديثة إلى أن له وظائف مهمة ترتبط بآليات عمل الأنسولين واستتباب الغلوكوز والأنسولين؛ وبناء عليه، قد يكون IDE واصماً حيوياً مفيداً في هذه الحالات. **تهدف هذه الدراسة** إلى مقارنة مستويات IDE المصلية بين أفراد مصابين بالمتلازمة الاستقلابية دون السكري نمط 2 (مجموعة المرضى)، وأشخاص أصحاء (مجموعة الشاهد) لدى عينة من البالغين السوريين. ودراسة علاقة مستويات IDE مع مكونات المتلازمة الاستقلابية وبعض القياسات الأنتروبومترية (البشرية) والسريرية والالتهابية والاستقلابية.

مواد البحث وطرائقه: شملت الدراسة 93 شخصاً من البالغين موزعين على مجموعتين: 45 شواهد أصحاء و48 مرضى. خضع كل مشارك لأخذ القصة المرضية وقياس بعض المتتبات البشرية (الأنتروبومترية) والسريرية والبدانة والالتهاب والمتتبات الاستقلابية.

النتائج: انخفضت مستويات IDE بشكل معتد به إحصائياً لدى المرضى مقارنة بالشواهد، وارتبطت سلبياً بالغلوكوز ($P > 0.05$). **الاستنتاجات:** يمكن أن يسهم الإنزيم المقوض للأنسولين في إمراضية المتلازمة الاستقلابية ويمكن أن تقيد مقايسته كواصم حيوي هام في هذه المتلازمة.

Background and Aim: Metabolic syndrome (MetS) forms a cluster of metabolic dysregulations including hyperglycemia and/or insulin resistance, atherogenic dyslipidemia, central obesity, and hypertension. MetS is a prominent cause of morbidity and mortality, and its etiology is believed to involve a complex interplay between genetic predisposition and environmental factors, especially insulin resistance. On the other hand, insulin degrading enzyme (IDE) is the main enzyme that degrades insulin. Recent studies suggested that it has important functions related to the mechanisms of insulin action, and insulin and glucose homeostasis; So it might be a useful biomarker in these cases. The aim of this study was to compare serum IDE levels between subjects having metabolic syndrome without type 2 diabetes mellitus (T2DM) (patient group) and healthy people (control group) in a sample of Syrian adults. And study the relationship of

IDE levels with the components of metabolic syndrome and some anthropometric, clinical, inflammatory and metabolic parameters.

Materials and Methods: 93 Syrian adult participants were divided into 2 groups (45 healthy controls and 48 patients). Personal history and measuring anthropometric, clinical, obesity, inflammatory, and metabolic parameters were applied to all participants.

Results: IDE levels were significantly lower in patients compared to the controls and negatively correlated with glucose ($P < 0.05$).

Conclusions: IDE might contribute to the pathogenesis of MetS, and its measurement may be useful as an important biomarker in this syndrome.

Key words الكلمات المفتاح

المتلازمة الاستقلابية MetS، الإنزيم المقوض للأنسولين IDE، السكري نمط ثاني T2DM، مقاومة الأنسولين.

Metabolic syndrome (MetS), insulin-degrading enzyme (IDE), type 2 diabetes mellitus (T2DM), insulin resistance.

Introduction

Metabolic syndrome, also termed syndrome X, insulin resistance syndrome, or Reaven syndrome, is a constellation of cardiometabolic risk factors including central obesity, insulin resistance, hyperglycemia, dyslipidemia, and elevated blood pressure (BP) [1]. It is a constellation of several chronic and inflammatory pathologies, which increase the risk of development of T2DM and its complications and atherosclerotic cardiovascular disease [2]. The global prevalence of metabolic syndrome has increased alongside rates of obesity over the past several decades, particularly in developed countries, and is considered a worldwide epidemic [3]. The incidence of MetS is expected to increase to approximately 53% in 2035 coinciding with the increasing prevalence of obesity [4, 5]. There are various hypothetical mechanisms regarding the pathophysiology of MetS; however, fatty acid flux, insulin resistance, low-grade chronic inflammation, genetic susceptibility, and oxidative stress are widely accepted as the crucial underlying factors involved in the initiation, progression, and transformation of MetS [6].

In 2009 the Joint Interim Statement approved a consensus to define MetS when any three of the following criteria are present: 1. High waist circumference (WC), whose thresholds depend on populations and country specific definitions (≥ 94 cm and ≥ 80 cm for men and women respectively for Europ, Eastern Mediterranean, and Middle East); 2. Blood TAG ≥ 150 mg/dL

and/or on drug treatment; 3. Blood HDL cholesterol < 40 mg/dL in men and < 50 in women and/or on drug treatment; 4. Blood pressure (BP) $\geq 130/85$ mmHg and/or on drug treatment; 5. Blood fasting glucose ≥ 100 mg/dL and/or on drug treatment [6, 7]. As MetS is closely linked to insulin resistance, biomarkers with a predictable association with insulin resistance might be useful to detect those at risk and intervene as needed. This could significantly decrease the burden complications impose on patients and the healthcare system. This is why it is important to highlight some unexplored features of the pathogenesis of MetS. The current study is one step in this context, focusing on the possible role of Insulin Degrading Enzyme (IDE) which is the main enzyme that degrades insulin, as a circulating biomarker contributing to the pathogenesis and disturbances in MetS.

Mirsky and Broh-Kahn discovered IDE in 1949, and it has since been characterized as neutral Zn^{2+} metalloprotease with a molecular weight of 110 k Da [8]. The IDE gene is located on human chromosome 10 q23-q25 and is synthesized as a single polypeptide [9]. IDE assembles as a stable homodimer where each monomer is comprised four homologous domains: The first two domains constitute the N-terminal portion (IDE-N), and the last two domains constitute the C-terminal portion (IDE-C). IDE-N and IDE-C are joined by an extended loop of 28 amino acids [10]. IDE is a major enzyme responsible for insulin degradation. In addition to insulin, IDE degrades many targets including glucagon, atrial natriuretic peptide, and beta amyloid peptide

[11]. It is a remarkable enzyme as it is expressed in all tissues, and is found in many subcellular environments, mainly in the cytosol, but also in endosomes, mitochondria, peroxisomes, nucleus, plasma membrane, and it is found extracellularly [12]. Numerous studies have found that IDE regulates liver and pancreas glucose transporters and hepatic insulin receptors levels, suggesting a non-proteolytic role of IDE in the regulation of intracellular trafficking of proteins involved in the regulation of insulin sensitivity and glucose tolerance [10, 13, 14].

The aim of this study is to investigate the variations in IDE levels between healthy and MetS without T2DM patients in a sample of Syrian adults; and to study their correlations to the components of the metabolic syndrome.

Materials and Methods

A case-control observational study was conducted at the Faculty of Medicine, Damascus University. Samples were collected from patients visiting endocrinology clinics at Al-Mouwasat Hospital from December 2022 to December 2023. The participants' agreements was obtained by informed consent and 93 adult participants were included. Participants were divided into two groups: control group (45 apparently healthy adults: 26 females and 19 males); and of group of patients (48 adults having metabolic syndrome without T2DM: 27 females and 21 males). MetS was diagnosed according to the Joint Interim Statement criteria [6]. The exclusion criteria of the study were: patients with diabetes mellitus, Pregnant women, subjects with, liver or kidney disease, endocrine disorders, acute or chronic infections, and subjects taking corticosteroids or sex steroids.

Anthropometric & clinical measurements were performed in the morning after 14-hour fasting according to the published protocols. Systolic and diastolic blood pressure (mmHg) were measured using a calibrated digital scale from HuBDIC (Korea) in the morning in a sitting position after resting, twice at an interval of 10 minutes, then the average of the two measurements was taken [5]. Weight (in

kilograms) was measured using a calibrated digital scale from LAICA (China) without shoes and in light clothing. Height (in meters) was measured, in a standing position using a tape measure [5]. Waist circumference was measured using a non-elastic tape placed around the waist midway between the lower costal margin and the top of the iliac bone. The tape level parallel to the floor with the person facing forward and feet together with both arms hanging freely. Measurements were taken three times and the average recorded [15]. Body mass index (BMI) (kg/m^2) was calculated by dividing the body weight (Kg) by the square of the height (meter) [16].

Laboratory measurements: 10 ml of venous blood was drawn in the morning after an overnight (14 hours) fast (for TG assay) into dry tubes. Serum was obtained and used directly for all measurements except for IDE, insulin and C-reactive protein CRP (the serum samples were stored at -20°C in the freezer in the blood bank at Al-Mowasat University Hospital until the measurements were performed. IDE was measured by Sandwich model of Enzyme-linked Immuno-sorbent Assay (ELISA) using a kit from Elabscience (USA) [17]. Serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), creatinine, albumin, and Alanine amino Transferase (ALT), were measured by colorimetric methods using kits from Mindray Co. (China) [18-21]. Insulin was measured by the latex-enhanced Immunoturbidimetric method using kit from Sekisui medical Co. LTD NORUDIA (Japan) [22], while CRP was measured by the immunoturbidimetric method using kit from DIASYS (Germany) [23]. The tests were conducted in the laboratories of Al-Mowasat University Hospital and Faculty of Medicine. LDL-C was calculated according to the Friedwald equation as follows: $\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TAG}/5)$ [24]. The homeostasis model was calculated to measure insulin resistance according to the homeostasis model assessment of insulin resistance (HOMA-IR) using the following equation: $\text{HOMA-IR} = \text{fasting glucose concentration (mg/dL)} \times \text{fasting insulin concentration (}\mu\text{U/mL)} \div 405$ [25]. **Statistical analysis** was done using SPSS version 22.0 (IBM Inc. software, Chicago,

USA). T-test was used to compare the means of the results that were expressed as mean \pm SD.

Pearson correlation was used to assess the correlation between parameters.

Results

Comparison of mean values

Table 1 shows the means, standard deviations, and statistical significance of the differences of the studied parameters between control and MetS groups. This table shows that the 2 groups are comparable with regard to the age and gender ($P > 0.05$). There were no statistically significant differences between control and MetS patients in serum albumin, TC, and LDL-C ($P = 0.14$, $P = 0.08$, $P = 0.17$ respectively). On the other hand, BMI, WC, SBP, DBP, and creatinine were significantly higher in MetS patients compared to controls ($P < 0.0001$, < 0.0001 , < 0.0001 , and < 0.001 , respectively). Levels of the studied glycemic parameters (FG, Insulin, and HOMA-IR) were significantly higher in MetS patients compared to controls ($P < 0.0001$ in all cases).

Regarding lipid profile, the results showed that HDL-C was lower in MetS patients compared to controls ($P = 0.016$), while TG was higher in MetS patients compared to controls ($P < 0.0001$) (Table 1). While There were no statistically significant differences between control and MetS patients in serum TC, and LDL-C ($P = 0.08$, $P = 0.17$ respectively) Inflammatory status, represented by CRP level (mg/l), was higher in MetS patients (6.30 ± 3.25) compared to the controls (2.03 ± 0.89) ($P < 0.0001$) (Table 1). The levels of IDE (ng/ml) were significantly lower in MetS patients (17.23 ± 4.64) compared to controls (21.80 ± 4.13) ($P < 0.0001$) (Table 1 and figure 1).

IDE correlations:

The only significant correlation between IDE and other studied parameters in patient group was the negative correlation between IDE and glucose ($r: -0.298$; $P < 0.05$).

Table (1): Anthropometric and biochemical parameters in control and Metabolic syndrome (MetS) groups.

	Control		MetS	
Number	45		48	
Females	26 (57.8%)		27 (56.25%) ^{NS}	
Males	19 (42.2%)		21 (43.75%) ^{NS}	
Parameters	M	SD	M	SD
Age (Years)	49.62	6.80	49.56	9.71 ^{NS}
BMI (Kg/m ²)	22.20	1.87	35.09	5.71 ^{A3}
WC(CM)	81.18	8.18	113.63	11.70 ^{A3}
SBP (mmHg)	109.27	9.95	134.98	13.43 ^{A3}
DBP (mmHg)	70.42	7.16	83.40	7.76 ^{A3}
Creatinine (mg/dl)	0.76	0.15	0.90	0.20 ^{A2}
Albumin (g/l)	4.62	0.44	4.48	0.46 ^{NS}
ALT(IU/l)	14.51	4.38	26.40	9.90 ^{A3}
FG(mg/dl)	84.93	7.93	103.75	19.56 ^{A3}
Insulin (μ U/ml)	7.78	2.74	17.32	6.62 ^{A3}
HOMA-IR	1.64	0.63	4.58	2.21 ^{A3}
TAG(mg/dl)	106.16	43.15	155.73	57.13 ^{A3}
TC (mg/dl)	176.53	26.01	189.67	43.65 ^{NS}
HDL-C (mg/dl)	47.56	12.01	41.85	10.56 ^{A1}
LDL-C (mg/dl)	107.76	24.65	116.67	37.11 ^{NS}
CRP (mg/l)	2.03	0.89	6.30	3.25 ^{A3}
IDE (ng/ml)	21.80	4.13	17.23	4.64 ^{A3}

Abbreviations: ALT: Alanine amino Transferase; BMI: body mass index; CRP: C-reactive protein; DBP: diastolic blood pressure; FG: fasting glucose; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; IDE: insulin-degrading enzyme; LDL-C: low-density lipoprotein cholesterol; M: Mean; SBP: systolic blood pressure; SD: standard deviation; TAG: triacylglycerols; TC: total cholesterol. WC: waist circumference.

Statistical significance (P value): NS: not significant; A1: $P < 0.05$; A2: $P < 0.001$; A3: $P < 0.0001$

Figure 1: Insulin degrading enzyme (IDE) among the studied groups.

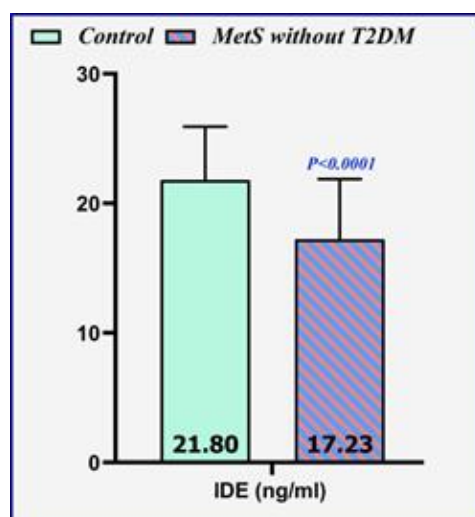


Figure (1): Insulin degrading enzyme (IDE) among the studied groups.

Discussion

It is not surprising to find, as shown in the present study, that BMI and WC are higher in MetS group compared to control one. These results are similar to that reported in previous studies and reviews [26-28]. It is well known that obesity, represented by increased BMI and WC, is associated with all other components of the metabolic syndrome. In the obese state, the adipocyte is integral to the development of obesity-induced inflammation by increasing secretion of various inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin1 (IL-1), and interleukin6 (IL-6), which in turn enhance reactive oxygen species (ROS) production, creating a vicious circle. Oxidative stress and inflammation are important components in the pathophysiology of obesity-related conditions such as atherosclerosis and insulin resistance [29].

The current study also showed that both SBP and DBP were higher in MetS patients compared to controls ($P<0.0001$). The elevation of blood pressure in MetS was reported by different studies [26-28]. It is well known that insulin resistance activates the sympathetic system, upregulates angiotensin II receptors, and reduces the synthesis of nitric oxide, leading to increases in heart rate and blood pressure [30]. Furthermore, increased actions of leptin, the presence of obstructive sleep apnea, and baroreflex dysfunction in MetS further contribute to the activation of the sympathetic system [31-33]. Also, in obese patients there is

an increase in renal tubular reabsorption with a consequent sodium retention, further contributing to the development of hypertension [34].

On the other hand, the present study shows that the levels of glycemic parameters (glucose, insulin, and HOMA-IR) are significantly higher in MetS patients compared to controls (Table 1). These results are in consistence with other studies showing that the levels of glucose [26-28, 35], insulin [26, 35, 36], and HOMA-IR are significantly higher in MetS patients compared to controls [26]. It is well known that elevated blood glucose is one of the diagnostic criteria of MetS and insulin resistance has been implicated in the development of MetS as it contributes to increased glucose production in the liver and decreased glucose uptake in the muscle, liver, and adipose tissue. All of these result in increased glucose concentration in the blood, and increased compensatory insulin production by pancreatic beta cells, which increases insulin concentration in the blood [37].

Regarding lipid profile, MetS patients have significantly higher levels of TG ($P<0.0001$) and lower levels of HDL-C ($P=0.016$) compared to controls. These results are in harmony with other studies [26-28, 36, 38]. On the other hand, There were no statistically significant differences between controls and MetS patients in serum TC and LDL-C (Table 1). These results are in harmony with those reported by Carmen Zaha et al [27], while they are not in consistence with those reported by Maleki et al [28]. This contradictory results may be due to the differences in the designs of these studies, the number of participants, the ratios of males to females, and the criteria used to diagnose metabolic syndrome [28]. It is well known that in obesity and insulin resistance increased free fatty acids (FFA) release from adipose tissue via lipolysis can result in enhanced delivery of FFA to the liver. The enhanced FFA leads to increase TG and very-low-density lipoprotein (VLDL) production in the liver, as well as inhibition of lipoprotein lipase in adipose tissue and skeletal muscle, thereby promoting dyslipidemia [39]. Insulin resistance also may cause low HDL cholesterol by several mechanisms: Diminished activity of lipoprotein lipase (LPL) may result in excessive transfer of TG from TG-rich chylomicrons and VLDL particles in exchange

for cholesterol esters from HDL particles thus reducing levels of HDL cholesterol and producing TG-rich HDL particles which form a substrate for hepatic lipase to be rapidly hydrolyzed resulting in a decrease in their circulating concentrations [40].

The present study also shows that the levels of CRP are significantly higher in MetS patients compared to controls ($P < 0.0001$). In the same line other studies showed that inflammatory status reflected by increased level of high-sensitivity C-reactive protein was significantly higher in MetS patients compared to controls [26, 28]. These results are in harmony with other studies provided that a chronic state of inflammation appears to be a central mechanism underlying the pathophysiology of insulin resistance and MetS [40, 41], and with those reported by Dallmeier et al and signified that MetS was associated with multiple inflammatory biomarkers. The authors also designated that the association between inflammation and MetS was largely accounted by MetS components [42]. It is well known that cytokines, mainly interleukin IL-1, IL-6, and tumor necrosis factor TNF- α , exert major stimulatory effects on hepatic synthesis of acute-phase proteins such as fibrinogen and CRP. Also, insulin resistance may lead to enhanced CRP expression [43, 44].

The levels of IDE in the current study were significantly lower in MetS patients compared to controls. IDE was significantly and negatively correlated with glucose. These findings suggest a role for IDE deficiency in the pathogenesis of metabolic syndrome and insulin resistance. This role can be explained by the proposed roles of IDE in glucose and insulin homeostasis, and in insulin signaling [10]. The findings of the present study are in harmony with those reported by Farris et al. They found that pancellular deletion of IDE in mice caused elevated fasting plasma glucose and insulin levels along with profound glucose intolerance [45]. Also the findings of the present study are in harmony with those reported by Abdul-Hay and colleagues who found that pancellular deletion of IDE in mice caused elevated fasting plasma insulin levels and age-dependent glucose and insulin intolerance [46]. Decreased levels of IDE in MetS patients in the current study

might be the cause of insulin resistance. These findings are in consistence with those reported by Najjar et al, and Villa-Pérez et al. They proposed that the main effect of IDE on insulin action appears to be mediated by its nonproteolytic activity. They proposed that hepatic ablation of IDE reduces both the levels and phosphorylation of insulin receptor leading to lower AKT (protein kinase B) activation which in turn enhances expression of gluconeogenic genes and cause hepatic insulin resistance [14, 47]. The results of the current study are consistent with the functions of IDE suggested in experimental studies which showed that the deletion of hepatic IDE resulted in decreased mRNA levels of glucose kinase and decreased glucose uptake by hepatocytes, causing hyperglycemia [48, 49]. These could explain the negative correlation of IDE in the present study with glucose. The results of the current study are also consistent with some experimental studies showed that loss of IDE function in diet-induced obese mice caused aggravation of insulin resistance and glucose intolerance, while enhancing IDE function had positive effects on glucose tolerance and improved insulin sensitivity [50]. In contrast, the findings of the present study were contradictory to those reported by Sofer et al. that found that IDE levels were higher in patients with metabolic syndrome than in controls ($P < 0.05$). This difference may be due to the differences in the study design, number of participants, the criteria used to diagnose MetS, and the assay used for measuring IDE. Sofer team of researchers used sandwich ELISA targeting an active conformation of IDE in the serum, while the current study relied on an ELISA method measuring total IDE levels in the serum [35].

Conclusions

The present study showed lower IDE values in a sample of Syrian adults having metabolic syndrome without T2DM compared to normal controls, and a negative association between these values and blood glucose.

Study limitations

The sample size is small and the study was limited to one center. So, these findings require conducting more large-scale studies to

understand the role of IDE in the pathogenesis of metabolic syndrome.

Ethics approval and consent to participate

Not applicable.

Conflict of interests

The authors declare that they have no competing interests.

Authors' contributions

SH carried out the laboratory works, collected the data and samples, and wrote the manuscript. YB performed the statistical analysis, participated in the design and coordination of the study and helped to edit the manuscript. ZA revised the manuscript for its substantive correctness. All authors read and approved the final manuscript.

Funding

This study was supported in part by Damascus University and the Ministry of High Education, Syrian Arab republic. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

References

1. Krishnaveni, G., S. Wagle, and C. Yajnik, *Intrauterine malnutrition and future risk of metabolic syndrome*, in *Metabolic Syndrome*. 2024, Elsevier. p. 21-34.
2. Satpathi, T., R. Unnikrishnan, and V. Mohan, *Intervening at the stage of metabolic syndrome to prevent type 2 diabetes—Is it justified?*, in *Metabolic Syndrome*. 2024, Elsevier. p. 35-43.
3. Burrage, L. and A. Sinha, *Understanding the complexities of metabolic syndrome in First Nations Australians*, in *Metabolic Syndrome*. 2024, Elsevier. p. 93-103.
4. Dong, S., et al., *Metabolic syndrome and breast cancer: prevalence, treatment response, and prognosis*. *Frontiers in oncology*, 2021. 11: p. 629666.
5. Belhayara, M.I., et al., *The metabolic syndrome: emerging novel insights regarding the relationship between the homeostasis model assessment of insulin resistance and other key predictive markers in young adults of Western Algeria*. *Nutrients*, 2020. 12(3): p. 727.
6. Jha, B.K., et al., *Progress in Understanding Metabolic Syndrome and Knowledge of Its Complex Pathophysiology*. *Diabetology*, 2023. 4(2): p. 134-159.
7. Desroches, S. and B. Lamarche, *The evolving definitions and increasing prevalence of the metabolic syndrome*. *Applied Physiology, Nutrition, and Metabolism*, 2007. 32(1): p. 23-32.
8. Tian, Y., G. Jing, and M. Zhang, *Insulin-degrading enzyme: roles and pathways in ameliorating cognitive impairment associated with Alzheimer's disease and diabetes*. *Ageing Research Reviews*, 2023: p. 101999.
9. Leissring, M.A., et al., *Targeting insulin-degrading enzyme in insulin clearance*. *International journal of molecular sciences*, 2021. 22(5): p. 2235.
10. González-Casimiro, C.M., et al., *Modulation of insulin sensitivity by insulin-degrading enzyme*. *Biomedicines*, 2021. 9(1): p. 86.
11. Pivovarova, O., et al., *Insulin-degrading enzyme: new therapeutic target for diabetes and Alzheimer's disease?* *Annals of medicine*, 2016. 48(8): p. 614-624.
12. Lesire, L., et al., *Insulin-Degrading Enzyme, an Under-Estimated Potential Target to Treat Cancer?* *Cells*, 2022. 11(7): p. 1228.
13. Binayi, F., et al., *Sustained feeding of a diet high in fat resulted in a decline in the liver's insulin-degrading enzyme levels in association with the induction of oxidative and endoplasmic reticulum stress in adult male rats: evaluation of 4-phenylbutyric acid*. *Heliyon*, 2024.
14. Najjar, S.M. and G. Perdomo, *Hepatic insulin clearance: mechanism and physiology*. *Physiology*, 2019. 34(3): p. 198-215.
15. Organization, W.H., *Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008*. 2011.
16. Mohajan, D. and H.K. Mohajan, *Body mass index (BMI) is a popular anthropometric tool to measure obesity among adults*.

- Journal of Innovations in Medical Research, 2023. 2(4): p. 25-33.
17. ELABSCIENCE. *Human IDE(Insulin Degrading Enzyme) ELISA Kit*. Available at: https://www.elabscience.com/p-human_ide_insulin_degrading_enzyme_elisa_kit-18196.html. 2023.
 18. Burtis, C.A. and E.R. Ashwood, *Tietz textbook of clinical chemistry*. Philadelphia, 1999. 1999: p. 1654-5.
 19. Rifai, N., P.S. Bachorik, and J.J. Albers, *Lipids, lipoproteins and apolipoproteins*. Tietz textbook of clinical chemistry, 1999. 3: p. 809-861.
 20. Kula, S.B., *Evaluation of Enzymatic Sarcosine Oxidase Method and Comparison with Modified Kinetic Jaffe's Reaction Analytical Method for Quantitative Analysis of Creatinine*. 2022, JKUAT-COHES.
 21. Thomas, L., *Clinical Laboratory Diagnostics*. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft Vol. 2. 1998.
 22. SEKISUI MEDICAL CO., L. *NORUDIA Isulin*. Retrieved from SEKISUI MEDICAL CO., LTD. 2017; Available from: international@sekisui.com.
 23. Hansson, L.-O. and L. Lindquist, *C-reactive protein: its role in the diagnosis and follow-up of infectious diseases*. Current Opinion in infectious diseases, 1997. 10(3): p. 196-201.
 24. Jagesh, R., et al., *Impact of Adoption of Directly Measured Low-Density Lipoprotein-Cholesterol (LDL-C) on Targets of Lipid Control and Its Comparison With Friedewald Formula-Calculated LDL Cholesterol in People With Type-2 Diabetes Mellitus*. Indian Journal of Clinical Cardiology, 2021. 2(3): p. 135-141.
 25. Wallace, T.M., J.C. Levy, and D.R. Matthews, *Use and abuse of HOMA modeling*. Diabetes care, 2004. 27(6): p. 1487-1495.
 26. Adams-Huet, B. and I. Jialal, *Correlates of insulin resistance in nascent metabolic syndrome*. Clinical Medicine Insights: Endocrinology and Diabetes, 2023. 16: p. 11795514231168279.
 27. Carmen Zaha, D., et al., *Influence of inflammation and adipocyte biochemical markers on the components of metabolic syndrome*. Experimental and therapeutic medicine, 2020. 20(1): p. 121-128.
 28. Maleki, A., et al., *Metabolic syndrome and inflammatory biomarkers in adults: a population-based survey in Western region of Iran*. International cardiovascular research journal, 2014. 8(4): p. 156.
 29. Masenga, S.K., et al., *Mechanisms of oxidative stress in metabolic syndrome*. International journal of molecular sciences, 2023. 24(9): p. 7898.
 30. Katsimardou, A., et al., *Hypertension in metabolic syndrome: novel insights*. Current hypertension reviews, 2020. 16(1): p. 12-18.
 31. Kotsis, V., et al., *Obesity and cardiovascular risk: a call for action from the European Society of Hypertension Working Group of Obesity, Diabetes and the High-risk Patient and European Association for the Study of Obesity: part B: obesity-induced cardiovascular disease, early prevention strategies and future research directions*. Journal of hypertension, 2018. 36(7): p. 1441-1455.
 32. Mancia, G., et al., *The sympathetic nervous system and the metabolic syndrome*. Journal of hypertension, 2007. 25(5): p. 909-920.
 33. Schlaich, M., et al., *Metabolic syndrome: a sympathetic disease? The lancet Diabetes & endocrinology*, 2015. 3(2): p. 148-157.
 34. Kotsis, V., et al., *Mechanisms of obesity-induced hypertension*. Hypertension research, 2010. 33(5): p. 386-393.
 35. Sofer, Y., et al., *Insulin-degrading enzyme higher in subjects with metabolic syndrome*. Endocrine, 2021. 71: p. 357-364.
 36. Lau, C.-H. and S. Muniandy, *Novel adiponectin-resistin (AR) and insulin resistance (IR AR) indexes are useful integrated diagnostic biomarkers for insulin resistance, type 2 diabetes and metabolic syndrome: a case control study*. Cardiovascular diabetology, 2011. 10: p. 1-18.
 37. Galicia-Garcia, U., et al., *Pathophysiology of type 2 diabetes mellitus*. International journal of molecular sciences, 2020. 21(17): p. 6275.
 38. Alexander, C.M., et al., *NCEP-defined metabolic syndrome, diabetes, and*

- prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes*, 2003. 52(5): p. 1210-1214.
39. Klop, B., J.W.F. Elte, and M. Castro Cabezas, *Dyslipidemia in obesity: mechanisms and potential targets*. *Nutrients*, 2013. 5(4): p. 1218-1240.
 40. Jung, U.J. and M.-S. Choi, *Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease*. *International journal of molecular sciences*, 2014. 15(4): p. 6184-6223.
 41. Welty, F.K., A. Alfaddagh, and T.K. Elajami, *Targeting inflammation in metabolic syndrome*. *Translational research*, 2016. 167(1): p. 257-280.
 42. Dallmeier, D., et al., *Metabolic syndrome and inflammatory biomarkers: a community-based cross-sectional study at the Framingham Heart Study*. *Diabetology & metabolic syndrome*, 2012. 4(1): p. 28.
 43. Festa, A., et al., *Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS)*. *Circulation*, 2000. 102(1): p. 42-47.
 44. De Feo, P., et al., *Physiological increments in plasma insulin concentrations have selective and different effects on synthesis of hepatic proteins in normal humans*. *Diabetes*, 1993. 42(7): p. 995-1002.
 45. Farris, W., et al., *Insulin-degrading enzyme regulates the levels of insulin, amyloid β -protein, and the β -amyloid precursor protein intracellular domain in vivo*. *Proceedings of the National Academy of Sciences*, 2003. 100(7): p. 4162-4167.
 46. Abdul-Hay, S.O., et al., *Deletion of insulin-degrading enzyme elicits antipodal, age-dependent effects on glucose and insulin tolerance*. *PloS one*, 2011. 6(6): p. e20818.
 47. Villa-Pérez, P., et al., *Liver-specific ablation of insulin-degrading enzyme causes hepatic insulin resistance and glucose intolerance, without affecting insulin clearance in mice*. *Metabolism*, 2018. 88: p. 1-11.
 48. Sousa, L., et al., *Insulin-degrading enzyme: an ally against metabolic and neurodegenerative diseases*. *The Journal of pathology*, 2021. 255(4): p. 346-361.
 49. Borges, D.O., et al., *Loss of postprandial insulin clearance control by Insulin-degrading enzyme drives dysmetabolism traits*. *Metabolism*, 2021. 118: p. 154735.
 50. Merino, B., et al., *Hepatic insulin-degrading enzyme regulates glucose and insulin homeostasis in diet-induced obese mice*. *Metabolism*, 2020. 113: p. 154352.