in vivo *38*: 975-981 (2024)
doi:10.21873/invivo.13530

Zinc Alpha-2 Glycoprotein, Acylated Ghrelin, and Zinc Levels in Prediabetics

EDA MERVE KURTULUŞ[1], DENIZHAN KARIŞ[2], ALEV MELTEM ERCAN[3] and DILDAR KONUKOĞLU[1]

[1]*Department of Medical Biochemistry, Cerrahpaşa Faculty of Medicine, İstanbul University-Cerrahpaşa, Istanbul, Turkey;*
[2]*Department of Biophysics, İstinye University, Faculty of Medicine, Istanbul, Turkey;*
[3]*Department of Biophysics, Cerrahpaşa Faculty of Medicine, İstanbul University-Cerrahpaşa, Istanbul, Turkey*

Abstract. *Background/Aim: Prediabetic stages of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) exhibit differences in the sites of insulin resistance. Serum Zinc α-2 glycoprotein (ZAG), acylated ghrelin (AG), and zinc (Zn) levels can affect IFG, IGT, and diabetic glucose tolerance (DGT) differently. This study examined the importance of ZAG, AG, and serum Zn levels in prediabetic individuals with IFG, IGT, and DGT, compared to those with normal glucose levels. Patients and Methods: The study was conducted at İstanbul University Cerrahpaşa-Cerrahpaşa Faculty of Medicine. A total of n=151 volunteers were classified according to the WHO criteria for diabetes after undergoing an oral glucose tolerance test. Plasma and serum samples were measured by Inductively Coupled Plasma Optical Emission Spectroscopy, ELISA, and immunoassay. Results: Prediabetic conditions became more prominent with the decrease in ZAG levels. ZAG levels showed a negative correlation with acylated ghrelin and Homeostatic Model Assessment for assessing beta-cell function and insulin resistance. Zinc levels were significantly lower in DGT. Conclusion: ZAG levels have regulatory effects on insulin resistance and plasma glucose levels are mediated by zinc and acylated ghrelin.*

Correspondence to: Eda Merve Kurtuluş, İstanbul Üniversitesi-Cerrahpaşa, Cerrahpaşa Tıp Fakültesi Yerleşkesi Kocamustafapaşa Cd. No: 53, Cerrahpaşa, 34098-Fatih/Istanbul, Turkey. E-mail: emkurtulus@gelisim.edu.tr

Key Words: Zinc alpha 2 glycoprotein, acylated ghrelin, zinc, oral glucose tolerance test, prediabetes.

Type 2 diabetes, preceded by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), is the most prevalent endocrine disease caused by insufficient insulin secretion or inefficiency. This not only leads to dysglycemia and overt diabetes but also to microvascular complications and cardiovascular disease (CVD) (2, 3). IFG and IGT exhibit disparities in the organ or tissue sites where insulin resistance manifests. IFG is related to hepatic insulin resistance with normal peripheral insulin sensitivity and a defect in insulin's early response to glucose. In contrast, IGT is predominantly associated with insulin resistance in the muscles and leads to a weakened response to postprandial insulin secretion (4). However, both of these abnormal dysglycemic states—solely termed as isolated IFG or isolated IGT, or when overlapped to form the grey zone as impaired fasting glucose accompanied with impaired postprandial dysglycemia—are all related to abnormalities in incretin hormones, glucagon dysregulation, high levels of inflammation, impaired lipolysis, and atherogenic dyslipidemia, and therefore, they should be studied separately (5). Adipose tissue, as an endocrine organ, releases bioactive peptides called adipokines (6). These adipokines are linked to insulin resistance, hyperglycemia, and adipocyte-released metabolites. Zinc-α2-glycoprotein (ZAG or AZGP1) is a recently identified soluble adipokine characterized by an open groove structure facilitating the binding of hydrophobic ligands, such as polyunsaturated fatty acids and zinc. This structural feature suggests a potential involvement in lipid mobilization processes, and a capacity to serve as a stimulator of uncoupling proteins (6). Although ZAG has been related to moderate weight loss *in vitro*, lipid mobilization, and cachexia in some studies, it is still unclear if serum ZAG levels are associated with obesity, metabolic syndrome, or insulin resistance (7). Some crystallographic studies have asserted that ZAG contains a strong affinity to zinc ions (8, 9), yet the relationship between serum ZAG protein and serum zinc (Zn) is not fully explained. Zn, however, is known for its relationship with insulin (10),

having antioxidant properties by protecting protein sulfhydryl groups (11). Furthermore, it contributes to the stabilization, storage, and release of insulin heterodimers and hexamers, thus playing a role in glycemic control (12). Another proposed role for Zn is its interaction with free fatty acids, which influences energy metabolism and insulin activity (13, 14).

Ghrelin is a unique lipolytic peptide hormone that also affects glucose metabolism (15). The 28-amino-residue peptide; acylated ghrelin (AG) which is the biochemically active form, is one of the multifunctional hormones that has been studied for its effects on sleep, behavior, as well as hunger and energy balance. Ghrelin, secreted from the oxyntic mucosa, is involved in the stimulation of lactotroph and corticotrope functions and pancreatic excretions (1). In some model studies, the effects of zinc treatment on AG levels in diabetic rats (16) and the regulation of mitochondrial lipid metabolism by AG were evaluated, but AG treatment was not proven to be beneficial (17). To evaluate the prediabetic importance of ZAG, AG, and zinc, these biomarkers were studied in control, diabetic, and prediabetic individuals with IFG and IGT.

Patients and Methods

The study was approved by the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (No: 201383045809/917) and was conducted following the Helsinki Declaration and Good Clinical Practice guidelines. All subjects provided their informed consent prior to participation. The study population consisted of volunteers who had registered for the first time at the Endocrinology Clinic Cerrahpaşa Faculty of Medicine, Istanbul, Turkey, for an oral glucose tolerance test (oGTT). Using the WHO criteria (18), groups were randomly formed from individuals with normal glycemia, impaired glucose tolerance (IGT), diabetic glucose tolerance (DGT), or Impaired Fasting Glucose Tolerance (IFG). Normal glycemia (NG) was defined as a fasting serum glucose level of <110 mg/dl and 2nd-h post-challenge serum glucose level of <140 mg/dl (n=23, 13 women, 10 men, mean age: 55.6±7.7 years). Subjects with fasting serum glucose levels less than 110 mg/dl were classified as IGT (n: 46, women: 26, men: 20, mean age: 58.2±8.4 years) if their 2nd-h post-challenge glucose levels were between 140 and 199 mg/dl, and as DGT (n: 30, women: 15, men: 15, mean age: 59.0±11.1 years) if their 2nd-h post-challenge serum glucose levels were ≥200 mg/dl. IFG subjects (n: 52, women: 29, men: 23, mean age: 56.2±7.2 years) had fasting blood glucose levels between 100 and 125 mg/dl and 2nd-h post-challenge serum glucose values less than 140 mg/dl. Exclusion criteria included hypertension, any cardiovascular complications, pregnancy, having a family history of diabetes, or being on diabetic medications.

Sample collection and preparation. After 12 h of fasting, 75 g of anhydrous glucose dissolved in 250 ml of water (oGTT) was given orally. Baseline and 2nd-h post-blood drawn were collected from the antecubital vein in EDTA-containing tubes or anticoagulant-free tubes. Blood samples were centrifuged within 20 min at 4˚C. All the serum and whole blood parameters (except ZAG, Zn, and ghrelin) were determined immediately. The Hitachi Modular P analyzer uses commercial kits to detect serum glucose, albumin, total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol levels (Modular D2400, Roche Diagnostic, Indianapolis, IN, USA). HPLC was used to determine HbA1C. Serum insulin levels were determined using a solid-phase-two-site chemiluminescent immunometric assay (Modular P800 Roche Diagnostics, GmbH, Mannheim, Germany). Serum samples were aliquoted and stored at −80˚C until being analyzed. Basal serum (0th h) ZAG and AG levels were assayed using a solid-phase sandwich ELISA (Human Zinc-alpha-2-glycoprotein Elisa Kit, Catalog No: E2245h, LOT:3G085C, Wuhan EIAab Science Co., Ltd, China, and Human Acylated Ghrelin ELISA, Wuhan EIAab Science Co., Ltd Catalog No: E8707h, LOT:3G085C, respectively). Serum ZAG and acylated ghrelin levels were expressed as ng per ml. Intra and inter-assay coefficients of variation (CV) for ZAG were 6.8 and 7.5%, respectively. Intra and inter-assay CV for AG were 6.5 and 7.0%, respectively.

Basal serum zinc concentrations were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES ThermoiCAP 6000 series; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 206,200 nm. Zinc levels were expressed as μg per dl. Height and body weight were measured with the patient standing in light clothes without shoes for the BMI calculation. Homeostatic model assessment (HOMA) for assessing beta-cell function and insulin resistance (HOMA-IR) was calculated by using the formula [fasting glucose (mg/dl)×fasting insulin (μU/ml)]/405 from the baseline blood. A post-hoc sample size analysis was conducted for the ANOVA test, which is utilized for groups with unequal sample sizes. Sample Size Calculator version 1.060 was employed, and a significance level (alpha) of 0.05 was set, resulting in a calculated test power of 0.82.

All data are expressed as means±standard deviation. Statistical significance was defined as $p<0.05$. Normality was tested and for parametric comparison within groups, ANOVA with post hoc Scheffe or Games-Howell test was used. For nonparametric comparison among groups, the pairwise Kruskal-Wallis test was used. For sex comparison, the Mann-Whitney *U*-test was used. ANCOVA was used to adjust for potential confounding factors, such as BMI and age. The relationship between the analyzed parameters was expressed using Spearman's rank correlation coefficients (p).

Results

Demographic details and baseline biochemical characteristics of the subjects are given in Table I. To address the prediabetic stages, groups' compliance with WHO criteria was examined. The NGT group's fasting blood glucose level was significantly lower than those of the IFG, IGT, and DGT groups (all $p<0.005$). The IGT group's fasting glucose level was significantly lower than those of the IFG and DGT groups (all $p<0.001$). 2nd hour blood glucose levels after OGTT in the DGT group were significantly higher than those in the NGT, IFG, and IGT groups (all $p<0.001$) and were higher in the IGT group, than in the NGT and IFG groups (both $p<0.001$).

HOMA-IR was significantly higher in the IFG group than in the NGT and IGT groups (both $p<0.001$). Furthermore, the HOMA-IR of the NGT group was significantly lower than those of the DGT and IGT groups ($p<0.001$ and $p<0.005$ respectively).

Table I. *Demographic details and biochemical characteristics of the subjects with normal (NGT), impaired (IGT), and diabetic (DGT) tolerance and the subjects with impaired fasting glucose tolerance (IFG).*

Groups	Normal glucose tolerance	Impaired fasting glucose	Impaired glucose tolerance	Diabetic glucose tolerance
n	23	52	46	30
Male/Female	10/13	23/29	21/25	15/15
Age (years)	55.6±7.7	56.2±7.2	58.2±8.4	59.0±11.1
Body Mass Index (kg/m^2)	28.2±7.3	31.0±3.4	30.8±5.8	31.0±3.2
Total cholesterol(mg/dl)	214.7±37.5	214.5±25.3	219.3±29.5	220.9±36.3
HDL-cholesterol (mg/dl)	52.7±8.1	48.9±9.1	46.7±9.6^{a2}	44.7±12.9^{a2}
LDL-cholesterol (mg/dl)	131.7±36.1	135.3±23.7	142.4±27.5	149.2±28.0
Triglycerides (mg/dl)	150.8±20.1	150.4±27.7	151.6±35.0	154.9±24.8
Fasting glucose (mg/dl)	92.7±7.5	110.1±5.7^{a1,c1}	98.6±7.2^{a2}	108.6±10.7^{a1,c1}
2nd hour OGTT (mg/dl)	116.3±16.1	112.6±21.0	165.4±17.7^{a1,b}	221.5±17.7^{a1,b,c1}
HOMA-IR	1.67±1.25	4.16±2.76^{a1,c1}	2.34±1.39^{a2}	3.04±1.78^{a1}
Albumin (g/dl)	4.5±0.17	4.60±0.81	4.28±1.71	4.22±0.44
CRP (mg/l)	3.92±5.57	5.05±3.99	5.40±6.10	7.59±6.79

[a]Comparison with NGT; [a1]p<0.001, [a2]p<0.05; [b]Comparison with IFG; [b]p<0.001; [c]Comparison with IGT; [c1]p<0.001, [c2]p<0.005.

Table II. *Mean of serum zinc alpha-2 glycoprotein (ZAG), acylated ghrelin (AG), and zinc levels of the subjects with normal (NGT), impaired (IGT), diabetic (DGT) tolerance and the subjects with impaired fasting glucose (IFG).*

Groups	NGT	IFG	IGT	DGT
ZAG (ng/ml)	59.79±9.50	42.73±14,71^{a1}	50.73±7.98^{a2,b2}	51.83±9.87^{a2,b2}
AG (ng/ml)	4.78±1.49	5.82±1.84^{a2}	6.82±1.62^{a1,b2,d1}	5.33±1.73^{a2}
Zinc (µg/dl)	113.5±24.2	122.4±25.4	108.2±19.8	92.6±23.4^{a2,b2,c1}

[a]Comparison with normal glucose tolerance; [a1]p<0.001, [a2]p<0.05; [b]Comparison with impaired fasting glucose; [b2]p<0.05; [c]Comparison with impaired glucose tolerance; [c1]p<0.05; [d]Comparison with diabetic glucose tolerance; [d1]p<0.05.

The mean age and the mean concentration values of baseline BMI, serum total cholesterol, LDL cholesterol (mg/dl), triglyceride (mg/dl), albumin (g/dl), CRP (mg/l) showed no variation among the groups (all p>0.05). HDL cholesterol levels (mg/dl) were lower in the IGT and DGT groups compared with the NGT group (p<0.005).

Serum ZAG, AG, and Zinc levels are given in Table II. The NGT group had significantly higher mean serum ZAG levels than the IFG, IGT, and DGT groups (p<0.001, p<0.05, and p<0.05, respectively). Furthermore, the mean serum ZAG level in the IFG group was significantly lower than those in the DGT and IGT groups (both p<0.05). Mean AG levels in the IGT group were significantly higher than those in the NGT, DGT, and IFG groups (p<0.001, p<0.05, and p<0.05, respectively). Mean serum zinc levels were significantly lower in the DGT group compared to those in the IFG, NGT, and IGT groups (p<0.05, p<0.05, and p<0.05 respectively). Serum zinc levels in the IFG group were higher than those in the IGT group, but no significant difference was observed. Only serum ZAG levels were found to be substantially different when groups were evaluated according to sex, as shown in Table III. Serum ZAG levels in males were higher in all groups (p<0.005).

Significant but weak to mild correlations were found among ZAG, ghrelin, Zn, and age, as shown in Table IV. Serum ZAG levels were negatively correlated with serum acylated ghrelin levels (r=–0.538, p<0.001), HOMA-IR levels (r=–0.342, p<0.001), and fasting glucose levels (r=–0.349, p<0.001). Serum ZAG levels were significantly negatively correlated with fasting glucose levels (r=–0.349, p<0.001), age (r=–0.171, p<0.05), and positively with 2nd-h glucose levels (r=0.187, p<0.05) and weakly with triglyceride levels (r=0.298, p<0.05). When split into NGT, IGT and IFG groups, only in the IFG group, there was a significant negative correlation between serum zinc levels and 2-h glucose levels (r=–0.367, p<0.001) and a positive correlation with ZAG concentration (r=0.523, p<0.001). And ghrelin showed a positive correlation with HOMA-IR (r=0.275, p<0.05).

To test the effect of BMI as a confounder, three subgroups were formed: normal (≤24; n=14), overweight (≥25-29; n=58), and obese (≥30; n=79). Only the DGT group's HOMA-IR level showed a significant difference in terms of

Table III. *Comparison of serum zinc alpha-2 glycoprotein (ZAG), acylated ghrelin, and zinc according to sex.*

Groups		NGT	IFG	IGT	DGT
HOMAIR	Male	1.53±1.18	3.78±2.39	1.88±0.73	2.61±1.78
	Female	1.79±1.29	4.45±3.03	2.44±1.22	3.47±1.73
Zn (μg/dl)	Male	113.30±30.18	119.86±22.47	106.04±20.98	91.60±29.87
	Female	113.76±19.81	124.37±27.77	109.96±18.93	93.66±14.53
ZAG (μg/ml)	Male	64.32±6.12*	43.03±16.06*	54.39±4.91*	54.42±12.06*
	Female	56.30±10.51	42.49±13.83	47.66±8.81	49.25±6.48
AGhrelin (ng/ml)	Male	4.392±1.49	5.99±1.92	6.65±1.42	5.34±1.77
	Female	5.07±1.50	5.69±1.78	6.95±1.79	5.32±1.74

Comparison to female subjects *$p<0.05$ with MWU test. NGT: Normal glucose tolerance; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; DGT: diabetic glucose tolerance.

BMI compared with the NGT group ($p=0.049$). To test for possible confounders, not only BMI but also age was tested through hierarchical regression; no interrelationship was discovered for age and BMI groups ($p=0.202$; $p=0.171$, and $p=0.382$; for the dual effect of Age+ BMI). A multivariate stepwise regression test revealed that no other factors were associated with HbA1c, except ZAG, which was shown to be independently (std coef. β=–0.432 and $p<0.001$) associated with HOMA-IR.

Discussion

In the stages leading up to diabetes, distinctive metabolic patterns emerge, contingent upon the location of insulin resistance—whether in the liver or peripheral tissues—specifications of the tissues involved in modulating glucose uptake (19). The meta-analysis of 16 studies with n=147,000 volunteers suggests that IFG and IGT groups should be addressed separately due to their distinctive pathophysiology (20). In individuals with isolated IFG, there is diminished sensitivity of β-cells and reduced insulin levels in the liver, rather than primary insulin resistance. Due to the elevated gluconeogenesis and impaired suppression of liver glucose output, high fasting glucose levels with high basal hyperinsulinemia occur. In isolated phases of IGT, there is notable insulin resistance in muscle tissue, along with compromised first- and second-phase insulin secretion from the pancreas. Additionally, β-cells exhibit reduced responsiveness to glucose levels. Consequently, following a carbohydrate-rich meal or glucose intake, muscle insulin resistance hinders glucose uptake, resulting in postprandial hyperglycemia (21, 22). In our study, we evaluated baseline serum ZAG, AG, and Zn levels, with their distinguished prediabetic significance, in isolated IFG, isolated IGT, and DGT groups.

Our first biomarker was ZAG, which is a soluble polypeptide adipokine that has been suggested to bind beta-3-adrenoreceptors (β-AR) in adipocytes (23). Russell *et al.* found that when ZAG and its β-AR antagonist (propranolol) were given to ob/ob mice, increased glucose excretion from urine, and glucose uptake into skeletal muscle was observed, which can regulate body weight and insulin sensitivity (24). ZAG expression and ZAG protein levels showed a negative correlation between insulin resistance in adults (25). In children negative correlation between ZAG and HOMA-IR, BMI, and fasting insulin levels were found (26). In our study, the HOMA-IR and serum ZAG levels showed a negative correlation (r=–0.342). Furthermore, mean blood ZAG levels in the NGT group were significantly higher than those in the FG, IGT, and DGT groups ($p<0.001$; $p<0.05$, and $p<0.05$, respectively), demonstrating that ZAG has an insulin-like effect by increasing glucose uptake by cells. Yang *et al.* and Qu *et al.*, also found that the control groups had the highest serum ZAG concentrations, while the type 2 diabetes mellitus group (T2DM) had the lowest ZAG levels (27). Qu *et al.*, also asserted that ZAG/HOMA-IR ratio is a better indicator of insulin resistance in prediabetics than the triglyceride/glucose ratio (28) but neither of these studies distinguish between IFG and IGT. Our results also showed that the mean serum ZAG levels in the IFG group were lower compared to the DGT and IGT groups ($p<0.05$, and $p<0.01$ respectively) indicating that ZAG has better potential

Table IV. *Pearson correlation analysis (r) between serum zinc alpha-2 glycoprotein (ZAG), acylated ghrelin, zinc, glucose, and HOMA-IR in the studied subjects.*

	Zinc	ZAG	Acylated ghrelin
ZAG	0.123	1	–0.538**
Acylated ghrelin	–0.159	–0.538**	1
HOMA-IR	0.043	–0.342**	0.127
Fasting glucose	0.005	–0.349**	0.017
2 hours of glucose	–0.367**	0.187*	–0.056
Age	–0.127	–0.171*	–0.217**
Triglyceride	0.161	0.298**	0.106

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

as a biomarker for hepatic insulin resistance. In our study, we took into consideration the influence of sex on serum ZAG levels. It was consistently observed that males exhibited higher ZAG levels across all groups. This finding aligns with Yeung *et al*.'s (29) who found that ZAG is independently associated with male sex, hyperglycemia, serum triglycerides, and C-reactive protein levels. In our study, ZAG levels were inversely related to hyperglycemia, positively correlated with serum triglycerides, and were greater in males.

Zinc is an essential trace element and is also involved in the synthesis, storage, secretion, conformational stability, and integrity of hexameric insulin (11). It has also been hypothesized that Zinc increases appetite-related gene expression (30). Insulin-like effects of zinc, such as lipogenesis and glucose transport have also been shown (31). In our study, mean serum zinc levels in the DGT group were significantly lower than those in the NGT, IFG, and IGT groups ($p<0.05$, $p<0.05$, and $p<0.05$, respectively). These low-serum Zn levels were just an indication of DGT and showed no correlation with HOMA-IR or triglycerides. While Zn exhibits insulin-mimetic properties, whether zinc is expended or recycled during glucose reuptake remains uncertain. Consequently, plasma and serum zinc levels do not reliably reflect the body's genuine zinc status. This leads to incongruities, wherein comparisons between insulin and zinc levels may yield disparate outcomes, manifesting as either diminished or baseline zinc levels (12, 31). One of our study's most notable findings was that serum levels of Zn and ZAG were only positively correlated in the IFG group ($p<0.001$). Even though this positive correlation might appear contradictory at first, we evaluate this as the utilization of serum zinc by both Zinc-α2-glycoprotein (ZAG) and insulin. This explains why zinc may mediate ZAG integrity, suggesting that decreased serum ZAG levels in the IFG group may be attributable to elevated levels of free Zn in the serum. Furthermore, BMI was higher in the IFG group than in the other groups, demonstrating that the link between ZAG and Zn also involves lipid metabolism. In the literature, Zn and free fatty acids bind to the same sites in ZAG (8, 32); hence, increased zinc concentrations decreased the binding of palmitate to ZAG, which is compatible with our findings. Ghrelin, the only known peptide that is modified by fatty s and octanoylated is also claimed to have an impact on adiposity and diabetes (33) and is also secreted from pancreatic alpha, beta, and epsilon cells (34). Acylated ghrelin promotes the regulation of feeding behavior, energy homeostasis, and adiposity (15, 32). In experimental settings, glucose administration or food intake decreases plasma AG concentrations (35, 36). Studies have shown that ghrelin has both stimulatory (36) and inhibitory effects (8, 37) on insulin secretion. In human subjects, insulin infusion also decreases AG concentrations (38) whereas parenteral administration of insulin does not affect AG concentrations (39). Our results revealed that basal AG levels, which is the active form of ghrelin (40) were higher in the prediabetic and diabetic stages, and the highest serum AG levels were found in the IGT group when compared with the IFG, DGT, and NGT groups ($p<0.001$, $p<0.050$, and $p<0.005$, respectively). AG levels in the IFG group were also higher than those in the NGT group ($p<0.05$). Although the mechanisms that govern AG secretion during fasting and postprandial suppression are unknown (41), our findings suggest that AG is more likely to indicate peripheral insulin resistance rather than hepatic insulin resistance. In our study, serum ZAG levels were also negatively correlated with serum AG levels. As a result, for the first time, we showed that serum Zinc and ZAG play a role in regulating acylated ghrelin metabolism independently of BMI (Figure 1).

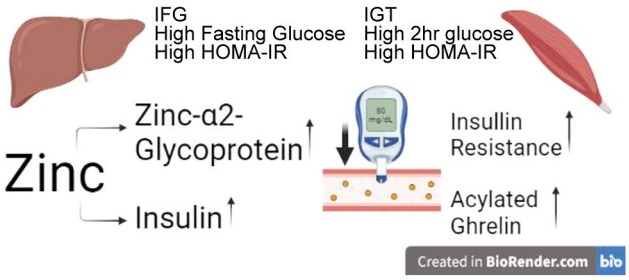

Figure 1. *Zinc alpha-2 glycoprotein, acylated ghrelin, and zinc levels in IFG and IGT groups.*

Conclusion

Our results show that when ZAG levels decrease, prediabetic conditions become prominent, and increased acylated ghrelin levels indicate a peripheral IR. As a result, ZAG may have regulatory effects on insulin resistance and on regulation of plasma levels by zinc and acylated ghrelin.

Funding

This work was supported by the Research Fund of Istanbul University. Project No. 29822.

Conflicts of Interest

The Authors declare no conflicts of interest in association with the present study.

Authors' Contributions

Eda Merve Kurtuluş: Conceptualization, Methodology, Analysis, Resources, Writing; Dildar Konukoğlu: Conceptualization, Methodology, Resources, Funding acquisition; Denizhan Karış: Analysis; Alev Meltem Ercan: Methodology.

References

1 Lindqvist A, Shcherbina L, Prasad RB, Miskelly MG, Abels M, Martínez-Lopéz JA, Fred RG, Nergård BJ, Hedenbro J, Groop L, Hjerling-Leffler J, Wierup N: Ghrelin suppresses insulin secretion in human islets and type 2 diabetes patients have diminished islet ghrelin cell number and lower plasma ghrelin levels. Mol Cell Endocrinol 511: 110835, 2020. DOI: 10.1016/j.mce.2020.110835

2 Cai X, Zhang Y, Li M, Wu JH, Mai L, Li J, Yang Y, Hu Y, Huang Y: Association between prediabetes and risk of all cause mortality and cardiovascular disease: updated meta-analysis. BMJ 370: m2297, 2020. DOI: 10.1136/bmj.m2297

3 Baranowska-Jurkun A, Matuszewski W, Bandurska-Stankiewicz E: Chronic microvascular complications in prediabetic states-an overview. J Clin Med 9(10): 3289, 2020. DOI: 10.3390/jcm9103289

4 Goyal R, Nguyen M, Jialal I: Glucose Intolerance. In: StatPearls. Treasure Island, FL, USA, StatPearls Publishing, 2022.

5 Holst JJ, Gasbjerg LS, Rosenkilde MM: The role of incretins on insulin function and glucose homeostasis. Endocrinology 162(7): bqab065, 2021. DOI: 10.1210/endocr/bqab065

6 Ceperuelo-Mallafré V, Ejarque M, Duran X, Pachón G, Vázquez-Carballo A, Roche K, Núñez-Roa C, Garrido-Sánchez L, Tinahones FJ, Vendrell J, Fernández-Veledo S: Zinc-α2-glycoprotein modulates AKT-dependent insulin signaling in human adipocytes by activation of the PP2A phosphatase. PLoS One 10(6): e0129644, 2015. DOI: 10.1371/journal.pone.0129644

7 Pearsey HM, Henson J, Sargeant JA, Davies MJ, Khunti K, Suzuki T, Bowden-Davies KA, Cuthbertson DJ, Yates TE: Zinc-alpha2-glycoprotein, dysglycaemia and insulin resistance: a systematic review and meta-analysis. Rev Endocr Metab Disord 21(4): 569-575, 2020. DOI: 10.1007/s11154-020-09553-w

8 Kumar AA, Hati D, Thaker TM, Miah L, Cunningham P, Domene C, Bui TT, Drake AF, McDermott LC: Strong and weak zinc binding sites in human zinc-α2-glycoprotein. FEBS Lett 587(24): 3949-3954, 2013. DOI: 10.1016/j.febslet.2013.10.026

9 Banaszak M, Górna I, Przysławski J: Zinc and the innovative Zinc-α2-glycoprotein adipokine play an important role in lipid metabolism: a critical review. Nutrients 13(6): 2023, 2021. DOI: 10.3390/nu13062023

10 Xu J, Zhou Q, Liu G, Tan Y, Cai L: Analysis of serum and urinal copper and zinc in Chinese northeast population with the prediabetes or diabetes with and without complications. Oxid Med Cell Longev 2013: 635214, 2013. DOI: 10.1155/2013/635214

11 Cruz KJC, De Oliveira ARS, Morais JBS, Severo JS, Mendes PMV, De Sousa Melo SR, De Sousa GS, Marreiro DDN: Zinc and insulin resistance: Biochemical and molecular aspects. Biol Trace Elem Res 186(2): 407-412, 2018. DOI: 10.1007/S12011-018-1308-Z

12 Wang X, Wu W, Zheng W, Fang X, Chen L, Rink L, Min J, Wang F: Zinc supplementation improves glycemic control for diabetes prevention and management: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 110(1): 76-90, 2019. DOI: 10.1093/ajcn/nqz041

13 Coverdale JPC, Khazaipoul S, Arya S, Stewart AJ, Blindauer CA: Crosstalk between zinc and free fatty acids in plasma. Biochim Biophys Acta Mol Cell Biol Lipids 1864(4): 532-542, 2019. DOI: 10.1016/j.bbalip.2018.09.007

14 Massih YN, Hall AG, Suh J, King JC: Zinc supplements taken with food increase essential fatty acid desaturation indices in adult men compared with zinc taken in the fasted state. J Nutr 151(9): 2583-2589, 2021. DOI: 10.1093/jn/nxab149

15 Deschaine SL, Leggio L: From "hunger hormone" to "it's complicated": Ghrelin beyond feeding control. Physiology (Bethesda) 37(1): 5-15, 2022. DOI: 10.1152/physiol.00024.2021

16 Bolkent S, Yanardag R, Bolkent S, Mutlu O, Yildirim S, Kangawa K, Minegishi Y, Suzuki H: The effect of Zinc supplementation on Ghrelin-immunoreactive cells and lipid parameters in gastrointestinal tissue of streptozotocin-induced female diabetic rats. Mol Cell Biochem 286(1-2): 77-85, 2006. DOI: 10.1007/s11010-005-9095-1

17 Barazzoni R, Bosutti A, Stebel M, Cattin MR, Roder E, Visintin L, Cattin L, Biolo G, Zanetti M, Guarnieri G: Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle. Am J Physiol Endocrinol Metab 288(1): E228-E235, 2005. DOI: 10.1152/ajpendo.00115.2004

18 World Health Organization and Federation ID: Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. World Health Organization, 2006. Available at: https://apps.who.int/iris/handle/10665/43588 [Last accessed on September 18, 2023]

19 Święcicka-Klama A, Połtyn-Zaradna K, Szuba A, Zatońska K: The natural course of impaired fasting glucose. Adv Exp Med Biol 1324: 41-50, 2020. DOI: 10.1007/5584_2020_571

20 Liu Y, Li J, Wu Y, Zhang H, Lv Q, Zhang Y, Zheng X, Tong N: Evidence from a systematic review and meta-analysis: Classical impaired glucose tolerance should be divided into subgroups of isolated impaired glucose tolerance and impaired glucose tolerance combined with impaired fasting glucose, according to the risk of progression to diabetes. Front Endocrinol (Lausanne) 13: 835460, 2022. DOI: 10.3389/fendo.2022.835460

21 Kanat M, Mari A, Norton L, Winnier D, DeFronzo RA, Jenkinson C, Abdul-Ghani MA: Distinct β-cell defects in impaired fasting glucose and impaired glucose tolerance. Diabetes 61(2): 447-453, 2012. DOI: 10.2337/db11-0995

22 Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA: Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance. Diabetes 55(5): 1430-1435, 2006. DOI: 10.2337/db05-1200

23 Bao Y, Bing C, Hunter L, Jenkins JR, Wabitsch M, Trayhurn P: Zinc-α_2-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes. FEBS Lett 579(1): 41-47, 2005. DOI: 10.1016/j.febslet.2004.11.042

24 Russell ST, Tisdale MJ: Antidiabetic properties of zinc-α2-glycoprotein in ob/ob mice. Endocrinology 151(3): 948-957, 2010. DOI: 10.1210/en.2009-0827

25 Chan GC, Than WH, Kwan BC, Lai KB, Chan RC, Teoh JY, Ng JK, Chow KM, Fung WW, Cheng PM, Law MC, Leung CB, Li PK, Szeto CC: Adipose and serum zinc alpha-2-glycoprotein (ZAG) expressions predict longitudinal change of adiposity, wasting and predict survival in dialysis patients. Sci Rep 12(1): 9087, 2022. DOI: 10.1038/s41598-022-13149-6

26 Barraco GM, Luciano R, Manco M: Zinc-α2-glycoprotein is associated with insulin resistance in children. Obesity 23(1): 5-6, 2015. DOI: 10.1002/oby.20948

27 Yang M, Liu R, Li S, Luo Y, Zhang Y, Zhang L, Liu D, Wang Y, Xiong Z, Boden G, Chen S, Li L, Yang G: Zinc-α2-

glycoprotein is associated with insulin resistance in humans and is regulated by hyperglycemia, hyperinsulinemia, or liraglutide administration: cross-sectional and interventional studies in normal subjects, insulin-resistant subjects, and subjects with newly diagnosed diabetes. Diabetes Care 36(5): 1074-1082, 2013. DOI: 10.2337/dc12-0940

28 Qu C, Zhou X, Yang G, Li L, Liu H, Liang Z: The natural logarithm of zinc-α2-glycoprotein/HOMA-IR is a better predictor of insulin sensitivity than the product of triglycerides and glucose and the other lipid ratios. Cytokine 79: 96-102, 2016. DOI: 10.1016/j.cyto.2015.12.024

29 Yeung DCY, Lam KSL, Wang Y, Tso AWK, Xu A: Serum zinc-α2-glycoprotein correlates with adiposity, triglycerides, and the key components of the metabolic syndrome in Chinese subjects. J Clin Endocrinol Metab 94(7): 2531-2536, 2009. DOI: 10.1210/jc.2009-0058

30 Suzuki H, Asakawa A, B. Li J, Tsai M, Amitani H, Ohinata K, Komai M, Inui A: Zinc as an appetite stimulator - the possible role of zinc in the progression of diseases such as cachexia and sarcopenia. Recent Pat Food Nutr Agric 3(3): 226-231, 2011. DOI: 10.2174/2212798411103030226

31 Attia JR, Holliday E, Weaver N, Peel R, Fleming KC, Hure A, Wiggers J, McEvoy M, Searles A, Reeves P, Ranasinghe P, Jayawardena R, Samman S, Luu J, Rissel C, Acharya S: The effect of zinc supplementation on glucose homeostasis: a randomised double-blind placebo-controlled trial. Acta Diabetol 59(7): 965-975, 2022. DOI: 10.1007/s00592-022-01888-x

32 Zahid H, Miah L, Lau AM, Brochard L, Hati D, Bui TT, Drake A, Gor J, Perkins S, McDermott L: Zinc-induced oligomerization of zinc α2 glycoprotein reveals multiple fatty acid-binding sites. Biochem J 473(1): 43-54, 2016. DOI: 10.1042/BJ20150836

33 Mani BK, Shankar K, Zigman JM: Ghrelin's relationship to blood glucose. Endocrinology 160(5): 1247-1261, 2019. DOI: 10.1210/en.2019-00074

34 Granata R, Settanni F, Biancone L, Trovato L, Nano R, Bertuzzi F, Destefanis S, Annunziata M, Martinetti M, Catapano F, Ghè C, Isgaard J, Papotti M, Ghigo E, Muccioli G: Acylated and unacylated Ghrelin promote proliferation and inhibit apoptosis of pancreatic β-cells and human islets: Involvement of 3',5'-cyclic adenosine monophosphate/protein Kinase A, extracellular signal-regulated Kinase 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling. Endocrinology 148(2): 512-529, 2007. DOI: 10.1210/en.2006-0266

35 Sato T, Nakamura Y, Shiimura Y, Ohgusu H, Kangawa K, Kojima M: Structure, regulation and function of ghrelin. J Biochem (Tokyo) 151(2): 119-128, 2012. DOI: 10.1093/jb/mvr134

36 Gupta D, Ogden SB, Shankar K, Varshney S, Zigman JM: "A LEAP 2 conclusions? Targeting the ghrelin system to treat obesity and diabetes". Mol Metab 46: 101128, 2021. DOI: 10.1016/j.molmet.2020.101128

37 Adeghate E, Ponery AS: Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. J Neuroendocrinol 14(7): 555-560, 2002. DOI: 10.1046/j.1365-2826.2002.00811.x

38 Lucidi P, Murdolo G, Di Loreto C, De Cicco A, Parlanti N, Fanelli C, Santeusanio F, Bolli GB, De Feo P: Ghrelin is not necessary for adequate hormonal counterregulation of insulin-induced hypoglycemia. Diabetes 51(10): 2911-2914, 2002. DOI: 10.2337/DIABETES.51.10.2911

39 Sovetkina A, Nadir R, Fung JNM, Nadjarpour A, Beddoe B: The physiological role of ghrelin in the regulation of energy and glucose homeostasis. Cureus 12(5): e7941, 2020. DOI: 10.7759/cureus.7941

40 Makris MC, Alexandrou A, Papatsoutsos EG, Malietzis G, Tsilimigras DI, Guerron AD, Moris D: Ghrelin and obesity: Identifying gaps and dispelling myths. A Reappraisal. In Vivo 31(6): 1047-1050, 2017. DOI: 10.21873/invivo.11168

41 Ennigrou S, Mami FB, Mahrez KB, Rayana CB, Slama FB: Study of serum ghrelin and resistin levels and their correlation with zinc and magnesium in men with type 2 diabetes. GSC Adv Res Rev 9(3): 070-083, 2021. DOI: 10.30574/gscarr.2021.9.3.0297

Received August 12, 2023
Revised September 17, 2023
Accepted September 18, 2023