

Resistin, Leptin In Human Seminal Plasma: Relationship With TNF-A And Parameters Semen In Oligozoospermia With And Without Varicocele

Hadi A. Abokallal, Department of Biology, College of Science, Al-Muthanna University, Samawa, Iraq

Nuha Yaarub Al-Harbi, Department of Biology, College of Science, University of Babylon, Hilla, Iraq.

Abstract: Purpose: *Varicocele may be associated with normozoospermic or oligozoospermia. Much dispute still exists regarding the diagnosis, management, and pathophysiology of spermatogenesis alterations associated with varicocele. We analyzed the two adipokines and tumor necrosis factor (TNF- α) in the seminal plasma of men with normozoospermic and oligozoospermia with or without varicocele.*

Materials and Methods: *Where the study performed in the Biology department, College of Science, University of Babylon, and an academic clinic in 70 consecutive patients with varicocele and 35 controls without varicocele. for the period of September 2019 to February 2020. The age of the patients was 21-50 years. The samples were divided according to WHO (2010) into two groups normozoospermia and oligozoospermia. There were 35 patients of men with primary infertility were diagnosed with varicocele and 35 without varicocele divided into four groups as oligozoospermia with varicocele (VO) (no. 13), oligozoospermia without varicocele (CO) (no. 21), and fertile controls as normozoospermic with varicocele (VN) (no.22) and normozoospermic without varicocele (CN) (no. 14)*

Results:

Increased seminal resistin and TNF- α ($P < 0.05$) levels were observed in the of men with varicocele and in those with normozoospermic and oligozoospermia. In this study reported adipokines levels in human seminal plasma in normal weight, overweight, and obese patients. seminal leptin and resistin level in overweight, and obese patients group was significantly higher ($P < 0.05$) than that in normal weight group, but there was significant difference in the seminal TNF- α levels between these two groups.

Keywords: *Male infertility; Adipokines; Tumor necrosis factor (TNF- α); Varicocele; Sperm quality.*

Introduction:

Varicocele, that is dilatation of veins along the spermatic cord with a backup of blood, is the most common recognizable abnormality in men evaluated for infertility. However, its role in damage spermatogenesis is still controversial. Varicocele is found in approximately 35% of men with primary infertility but also in about 15% of the general population and it may be associated with normal fertility (1). The vast generality of studies showed the negative effects of varicocele on fertility are widely known. Researchers have shown that varicocele-mediated spermatozoa damage might be the main cause of male infertility is directly or indirectly responsible for 60% of cases involving the conditions that compromise male fertility potential reproductive-age couples (2). pathophysiological have an effect on of varicocele on spermatogenesis and sperm features have been fully investigated recently. Many mechanisms

have been proposed, the most frequent of which used to be the increased oxidative stress which has a negative impact via many methods such as causing sperm DNA damage, mitochondrial defects, and growing sperm apoptosis (3).

In previous studies on obesity, different variables have been found to be connected to Obesity is described by an increased number of adipose cells and excessive storage of triglycerides in the adipose cells. The hormonal interaction between the adipose tissue and other endocrine organs including the gonads is complicated and not fully understood. Some endocrine changes involving adipokines could contribute to understanding the negative effects of obesity on reproductive function (4). Adipose tissue acts as endocrine (5). It is secreted adipokines These adipokines are not only synthesized and secreted at most by adipocytes but also synthesized and secreted by the other cells that pretend the adipose tissue, such as macrophages, lymphocytes, and fibroblasts (6) Previous research has decided that proinflammatory cytokines are secreted mainly by nonadipose cells in adipose tissue (7).

Adipokines, such as leptin, resistin, adiponectin, chemerin, omentin, and visfatin. Data from different sources have particular they are implicated in functions of the organism including those relevant to the gonadal and hypothalamic-pituitary axis, both in females and in males (8). Many studies demonstrated that leptin and resistin are present in the male reproductive tract and may be important in the pathogenesis of impaired spermatogenesis (9). tumor necrosis factor- (TNF and interleukin-6 (IL-6) (10). These cytokines are regulatory peptides generated and secreted by leukocytes and other cells and they have been implicated as growth and recognition factors (11). The seminal plasma contains several cytokines, which are normally present in the male genital tract. TNF- stimulate sperm peroxidation by increased reactive oxygen species (ROS) generation and apoptosis (12).

Materials and Methods:

Patients:

We analyzed were 35 patients of men with primary infertility were diagnosed with varicocele and 35 without varicocele divided into four groups as oligozoospermia with varicocele (VO), oligozoospermia without varicocele (CO), and fertile controls as normozoospermic with varicocele (VN) and normozoospermic without varicocele (CN) The samples were divided according to WHO (2010)(13). The age of the patients was 21-50 years the evaluation of the patients included information about their medical history, lifestyle factors such as the use of cigarettes, drugs, and alcohol, clinical and physical examinations and scrotal Eco-color Doppler to explore the possible presence of varicocele.

Resistin, and TNF- α Measurements: Leptin

Leptin, Resistin and TNF- α were assayed in semen samples of 70 recovered 1 hour after collection and fractioned by centrifugation (1,500 rpm for 15 minutes). The supernatant , individuals composed of seminal plasma without spermatozoa, was immediately stored at 80C until analyses were performed.

Resistin levels were determined by ELISA using the Resistin (Human) ELISA kit (bt-laboratory,China). Following the manufacturer's instructions, the results were expressed in nanograms per liter.

Statistical Analysis:

Statistical analysis was carried out using SPSS version 23, where data were expressed as the data were reported as mean and SE, and the normality of distribution was tested using the Levene test was performed to assess the homogeneity of variances. The independent t-test was used for comparisons between sperm parameters and seminal levels of leptin, resistin, and TNF was used to find the association between the categorical variables and between P-value ($P \leq 0.05$) was considered statistically significant. The Pearson correlation coefficient was calculated for the bivariate correlation analysis.

Results and Discussion:

Patient characteristics

The spermatozoa from a total of 70 patients were analyzed. Of these, 35 patients of men with primary infertility were diagnosed with varicocele and 35 without varicocele divided into four groups as oligozoospermia with varicocele (VO) (no.13), oligozoospermia without varicocele(CO) (no.21), and fertile controls as normozoospermic with varicocele (NV) (no.22) and normozoospermic without varicocele (CN) (no.14). Routine semen analysis results are shown in (Table 1& 2). Using routine sperm analysis, we compared volume, Total sperm count, Sperm concentration, Sperm progressive motility (%), and Total motility between the CN, VN, and VO. The average age of the normozoospermic varicocele (VN) and normozoospermic without varicocele (CN) in this study was (30.84±1.42), (31.84±1.60) years no differences between them. In this study, patients' mean age was (25.30±2.88) of patients were VO. There was no statistically significant difference between VO patients versus CN (p=0.779), also non-difference between VO and CO (p=0.305) for age. This corresponds to canals et al. (2005) they revealed did not demonstrate an age-related increase in varicocele prevalence (14) there was no statistical significant difference (p=0.046, > 0.05) in BMI between VO and CN groups, also between VO and CO (P= 0.312) groups. There was no statistically significant difference (p=0.096, > 0.05) in BMI between CN and VN groups (Table1), also between VO and CN groups (Table2) (p= 0.346), Nor between VO and CO groups (P=0.312) (Table3). we have agreed with some studies have found no significant differences in BMI between men with and without varicocele(15)& (16).

The values of semen volume were non significantly differencing in the three groups VO (2.20±0.15), VN (2.50±0.22) and CO (2.69±0.20). This finding is identical to previous studies which have suggested that there was no significant difference in the semen volume values after varicocele repair (17). When total sperm count was analyzed, the VN (197.73±20.14) was significantly lower than the CN (121.43±33.42) group (P=0.049); the percentage of progressive motility, and the total sperm motility, total sperm count and total sperm concentration were significantly reduced in the group of VO patients (P = 0:000) compared to those observed in the CN group (Table 2). in varicocele patients associated with low sperm count, sperm morphology, and motility (18). The etiology and pathophysiology of varicocele are multifactorial these varicoceles participate in low sperm counts and varicocelectomy can slightly restore spermatogenesis and fertility (19). Results of this study demonstrate that the presence of varicocele, in oligozoospermia, was associated with a decrease in the percentages of spermatozoa with total sperm count, motility, and progressive compared with normozoospermic without varicocele group. Comparing the result: supporting previous findings that suggest that varicocele affects sperm quality (20). These results are, therefore, consistent in suggesting a negative association between varicocele and sperm motility (21).

Sperm analysis and seminal plasma leptin, Resistin and TNF- α assays:

The seminal plasma leptin levels no significant differences between VN, VO, and CN groups (0.28±0.03), (0.38±0.07), and (0.31±0.02). No significant difference between CO (0.31±0.06) and VO (0.38±0.07) for leptin levels also Previously, many studies were carried out to determine the role between leptin and male infertility, however, no data revealed the relationship of Leptin concentrations with male infertility have been reported(22) & (23).

Seminal plasma resistin was higher in the VN (0.38±0.04) group when compared with the CN (0.15±0.01) group. These differences were statistically significant (P = 0.000, <0.05), and the results are shown in Table 1. In our study, we observed high resistin between groups VO and CN (Table 2), accompanied by a decrease in the total number and sperm motility. Our

results were consistent with the findings of (Moretti *et al.* (2014) showed that there was a negative correlation between the concentrations of seminal resistin and spermatic motility and vitality(24), also seminal plasma TNF- was statistically significant increase in the VN (0.32 ± 0.02) and VO (0.31 ± 0.04) groups when compared with the CN(0.20 ± 0.02)($P < 0.05$). Many studies showed the subfertile men had higher seminal resistin and TNF levels. Resistin is an adipokine able to excite proinflammatory state in vitro and in vivo and to play a role in stimulating the expression of other cytokines (25). These results corroborate the findings of the previous work who's confirmed that TNF- α levels are increased in the seminal plasma of oligozoospermic compared to control patients (26).

In the patients having varicocele oligozoospermia leptin concentrations (0.38 ± 0.07 ng/ml) As for the resistin it has a clear significance increase, as it was in group VO (0.40 ± 0.10) higher than CO (0.17 ± 0.01), ($p = 0.004$) (Table 3). Prior studies have noted the non-statistically significant (p -value > 0.05) correlations between seminal leptin levels before and after varicocelectomy (27). Other studies showed a significant relationship between resistin and pro-inflammatory cytokines was demonstrated in human semen samples (24). macrophages and neutrophils are both major sources of the resistin interested in the inflammatory pathway. The varicocele induces inflammation and then, impairs spermatogenesis (28 & 29). Several reports have shown that the seminal plasma TNF- α levels in patients with varicocele were higher than those in the control group, and that seminal leptin levels are unrelated in these patients (30).

Table (1): Clinical and laboratory data for Control normozoospermic and Varicocele normozoospermic groups (means \pm SD)

Parameters	Control normozoospermia (CN) Means \pm SD	Varicocele normozoospermia (VN) Means \pm SD	P-Value
Age(years)	31.84\pm1.60	30.84\pm1.42	0.564
BMI(Km/m ²)	26.54\pm0.913	28.89\pm0.84	0.096
Volume(ml)	3.06\pm0.29	2.50\pm0.22	0.183
Total sperm count (million/ml)	197.73\pm20.14	121.43\pm33.42	0.049
Sperm concentration(million/ml)	67.75\pm6.37	52.27\pm13.54	0.255
Sperm progressive motility (%)	55.33\pm1.46	49.09\pm3.80	0.085
Total sperm motility(%)	55.44\pm4.45	55.00\pm2.69	0.946
Seminal leptin(ng/L)	0.31\pm0.02	0.28\pm0.03	0.471
Seminal resistin(ng/L)	0.15\pm0.01	0.38\pm0.04	0.000
Seminal TNF- α (ng/L)	0.20\pm0.02	0.32\pm0.02	0.002

Table (2): Clinical and laboratory data for Control normozoospermia and Varicocele oligozoospermic groups (means±SD)

Parameters	Control normozoospermia (CN) Means ±SD	Varicocele oligoasthenozoospermia (VO) Means ±SD	P-Value
Age (years)	31.84±1.60	25.30 ±2.883	0.779
BMI (Km/m ²)	26.54±0.91	27.85±1.30	0.346
Volume (ml)	3.06±0.29	2.20±0.15	0.063
Total sperm count (million/ml)	192.584±19.730	18.50±3.383	0.000
Sperm concentration (million/ml)	66.815±6.097	8.00±1.154	0.000
Sperm progressive motility %	55.57±1.40	15.50±3.83	0.000
Total sperm motility (%)	61.47± 1.50	30.50±2.29	0.000
Seminal leptin (ng/L)	0.31±0.02	0.38±0.07	0.191
Seminal resistin(ng/L)	0.15±0.01	0.40±0.10	0.002
Seminal TNF- α (ng/L)	0.20± 0.02	0.31±0.04	0.007

Table (3): Clinical and laboratory data for Control normozoospermia and Varicocele oligozoospermic groups (means±SD)

Parameters	Control oligoasthenozoospermia (CO) Means ±SD	Varicocele oligoasthenozoospermia (VO) Means ±SD	P-Value
Age(years)	31.111±2.04	25.30 ±2.88	0.305
BMI(Km/m ²)	26.26±1.15	27.85±1.30	0.312
Volume(ml)	2.69±0.20	2.20±0.15	0.107
Total sperm count (million/ml)	23.91± 3.23	18.50±3.38	0.291
Sperm concentration(million/ml)	9.01± 1.13	8.00±1.15	0.723

Sperm progressive motility (%)	17.66± 2.82	15.50±3.83	0.621
Total sperm motility (%)	30.18± 4.34	30.50±2.29	0.838
Seminal leptin (ng/ml)	0.31± 0.06	0.39± 0.07	0.564
Seminal resistin (ng/ml)	0.17±0.01	0.40±0.10	0.004
Seminal TNF-α (ng/L)	0.19±0.02	0.31±0.04	0.025

Body Max Index, Leptin, Resistin and TNF- α :

Through our study, the relationship between adipokines and TNF- α was recorded during weight after group splitting into Normal weight and Obese/ Overweight in patients with varicocele only who are in group VN and VO. The WHO defines overweight and obesity when the BMI is ≥ 25 kg/m² and ≥ 30 kg/m², respectively (31).

Table (4): The means of Leptin, Resistin, TNF- α in varicocele subfertile men according BMI.

Parameters	VN (Varicocele normozoospermia)			Varicocele oligozoospermia (OV)		
	Normal weight Means \pm SD	Obese/ Overweight Means \pm SD	P- Value	Normal weight Means \pm SD	Obese/ Overweight Means \pm SD	P- Value
Leptin(ng/L)	0.16 \pm 0.03	0.36 \pm 0.02	0.002	0.09 \pm 0.02	0.34 \pm 0.07	0.047
Resistin(ng/L)	0.12 \pm 0.03	0.38 \pm 0.04	0.014	0.19 \pm 0.02	0.45 \pm 0.09	0.053
TNF- α (ng/L)	0.17 \pm 0.05	0.37 \pm 0.01	0.000	0.22 \pm 0.01	0.36 \pm 0.03	0.029

Thus, comparing two groups Normal weight versus Obese/ Overweight the following was observed in the VN significant increase of leptin (0.36 \pm 0.02), Resistin (0.38 \pm 0.04), and TNF- α (0.37 \pm 0.01) in the Obese/ overweight group compared to the normal weight. Also are reported to have a significant increase of seminal leptin (0.34 \pm 0.07), seminal resistin (0.45 \pm 0.09) and TNF- α (0.36 \pm 0.03) in VO of the Obese/ Overweight compared to Normal weight group. These results are in accordance with the majority of previously published data of Obese individuals to have higher circulating leptin levels as well as a higher prevalence of infertility (32). This supported the presence of a link between varicocele induced spermatogenesis dysfunction and elevated seminal and serum leptin (33). Leptin is a key regulator of fat storage and maybe directly participatory in sperm function, possibly serving as a link between obesity and reproductive dysfunction (34). Also, Adipose tissue releases chemokines like adiponectin, visfatin, resistin, tumor necrosis factor (TNF)- α , interleukin6 and their balance is dysregulated in obesity (35). Other research emphasizes the coordinated roles of resistin, adiponectin in the modulation of the pro-inflammatory environment observed in obese and morbidly obese patients (36).

In conclusion, In conclusion, thus adipokine profiles in seminal plasma could be a biomarker of male fertility. It could be interesting to measure these markers in the semen of subfertile men in varicocele patients and without varicocele. It is Clear that adipokines might be a link between obesity and male infertility.

References:

- [1] Jarow, J. P. (2001). Effects of varicocele on male fertility. *Human Reproduction Update*, 7(1), 59-64.
- [2] Sharlip, I. D., Jarow, J. P., Belker, A. M., Lipshultz, L. I., Sigman, M., Thomas, A. J., ... & Overstreet, J. W. (2002). Best practice policies for male infertility. *Fertility and sterility*, 77(5), 873-882.
- [3] Pasqualotto, F. F., Sundaram, A., Sharma, R. K., Borges Jr, E., Pasqualotto, E. B., & Agarwal, A. (2008). Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. *Fertility and sterility*, 89(3), 602-607.
- [4] Dupont, J., Pollet-Villard, X., Reverchon, M., Mellouk, N., & Levy, R. (2015). Adipokines in human reproduction. *Hormone Molecular Biology and Clinical Investigation*, 24(1), 11-24.
- [5] Coelho, M., Oliveira, T., & Fernandes, R. (2013). Biochemistry of adipose tissue: an endocrine organ. *Archives of medical science: AMS*, 9(2), 191.
- [6] Thomas, S., Kratzsch, D., Schaab, M., Scholz, M., Grunewald, S., Thiery, J., ... & Kratzsch, J. (2013). Seminal plasma adipokine levels are correlated with functional characteristics of spermatozoa. *Fertility and sterility*, 99(5), 1256-1263.-
- [7] Antuna-Puente, B., Feve, B., Fellahi, S., & Bastard, J. P. (2008). Adipokines: the missing link between insulin resistance and obesity. *Diabetes & metabolism*, 34(1), 2-11.
- [8] Acquarone, E., Monacelli, F., Borghi, R., Nencioni, A., & Odetti, P. (2019). Resistin: a reappraisal. *Mechanisms of ageing and development*, 178, 46-63.
- [9] Kratzsch, J., Paasch, U., Grunewald, S., Mueller, M. A., Thiery, J., & Glander, H. J. (2008). Resistin correlates with elastase and interleukin-6 in human seminal plasma. *Reproductive biomedicine online*, 16(2), 283-288.
- [10] Degelmann, A., Müller, F., Sieber, H., Jenzelewski, V., Suckow, M., Strasser, A. W., & Gellissen, G. (2002). Strain and process development for the production of human cytokines in *Hansenula polymorpha*. *FEMS yeast research*, 2(3), 349-361.
- [11] Martínez, P., Proverbio, F., & Camejo, M. I. (2007). Sperm lipid peroxidation and pro-inflammatory cytokines. *Asian journal of andrology*, 9(1), 102-107.
- [12] Perdichizzi, A., Nicoletti, F., La Vignera, S., Barone, N., D'Agata, R., Vicari, E., & Calogero, A. E. (2007). Effects of tumour necrosis factor- α on human sperm motility and apoptosis. *Journal of clinical immunology*, 27(2), 152-162.
- [13] Camejo, M. I., Abdala, L., Vivas-Acevedo, G., Lozano-Hernández, R., Angeli-Greaves, M., & Greaves, E. D. (2011). Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters. *Biological trace element research*, 143(3), 1247-1254.
- [14] Canales, B. K., Zapzalka, D. M., Ercole, C. J., Carey, P., Haus, E., Aeppli, D., & Pryor, J. L. (2006). Prevalence and Effect of Varicoceles in an Elderly Population. *The Journal of Urology*.
- [15] Delaney, D. P., Carr, M. C., Kolon, T. F., Snyder III, H. M., & Zderic, S. A. (2004). The physical characteristics of young males with varicocele. *BJU international*, 94(4), 624-626.
- [16] Kılıç, S., Aksoy, Y., Sincer, İ., Oğuz, F., Erdil, N., & Yetkin, E. (2007). Cardiovascular evaluation of young patients with varicocele. *Fertility and sterility*, 88(2), 369-373.
- [17] Vivas-Acevedo, G., Lozano-Hernández, R., & Camejo, M. I. (2014). Varicocele decreases epididymal neutral α -glucosidase and is associated with alteration of

- nuclear DNA and plasma membrane in spermatozoa. *BJU international*, 113(4), 642-649.
- [18] Camejo, M. I., Abdala, L., Vivas-Acevedo, G., Lozano-Hernández, R., Angeli-Greaves, M., & Greaves, E. D. (2011). Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters. *Biological trace element research*, 143(3), 1247-1254.
- [19] Zini, A., Blumenfeld, A., Libman, J., & Willis, J. (2005). Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. *Human Reproduction*, 20(4), 1018-1021.
- [20] Andrade-Rocha, F. T. (2007). Significance of sperm characteristics in the evaluation of adolescents, adults and older men with varicocele. *Journal of postgraduate medicine*, 53(1), 8.
- [21] Abd-Elmoaty, M. A., Saleh, R., Sharma, R., & Agarwal, A. (2010). Increased levels of oxidants and reduced antioxidants in semen of infertile men with varicocele. *Fertility and sterility*, 94(4), 1531-1534.
- [22] Zorn, B., Osredkar, J., Meden-Vrtovec, H., & Majdic, G. (2007). Leptin levels in infertile male patients are correlated with inhibin B, testosterone and SHBG but not with sperm characteristics. *International journal of andrology*, 30(5), 439-444.
- [23] Hanafy, S., Halawa, F. A., Mostafa, T., Mikhael, N. W., & Khalil, K. T. (2007). Serum leptin correlates in infertile oligozoospermic males. *Andrologia*, 39(5), 177-180.
- [24] Moretti, E., Collodel, G., Mazzi, L., Campagna, M., Iacoponi, F., & Figura, N. (2014). Resistin, interleukin-6, tumor necrosis factor-alpha, and human semen parameters in the presence of leukocytospermia, smoking habit, and varicocele. *Fertility and sterility*, 102(2), 354-360.
- [25] Filková, M., Haluzík, M., Gay, S., & Šenolt, L. (2009). The role of resistin as a regulator of inflammation: Implications for various human pathologies. *Clinical immunology*, 133(2), 157-170.
- [26] Haney, A. F., HUGHES, S. F., & WEINBERG, J. B. (1992). The lack of effect of tumor necrosis factor-alpha, interleukin-1-alpha, and interferon-gamma on human sperm motility in vitro. *Journal of andrology*, 13(3), 249-253.
- [27] Elshahid, A. R., Al-Adl, A. S., Esmail, N. F., & Elsayed, M. I. Measurement of Leptin Hormone in Serum and Seminal Plasma Before & After Varicocelectomy.
- [28] Schwartz, D. R., & Lazar, M. A. (2011). Human resistin: found in translation from mouse to man. *Trends in Endocrinology & Metabolism*, 22(7), 259-265.
- [29] Moretti, E., Cosci, I., Spreafico, A., Serchi, T., Cuppone, A. M., & Collodel, G. (2009). Semen characteristics and inflammatory mediators in infertile men with different clinical diagnoses. *International journal of andrology*, 32(6), 637-646.
- [30] Mrkun, J., Kosec, M., & Zrimšek, P. (2013). Value of semen parameters, with special reference to tnf- α , in predicting the quality of boar semen after short-term storage. *Acta Veterinaria Hungarica*, 61(2), 209-219.
- [31] World Health Organization. (2016). World Health Organization obesity and overweight fact sheet.
- [32] Kasturi, S. S., Tannir, J., & Brannigan, R. E. (2008). The metabolic syndrome and male infertility. *Journal of andrology*, 29(3), 251-259.

- [33] Zhang, J., Jin, P. P., Gong, M., Yi, Q. T., & Zhu, R. J. (2018). Role of leptin and the leptin receptor in the pathogenesis of varicocele-induced testicular dysfunction. *Molecular medicine reports*, 17(5), 7065-7072.
- [34] Guo, J., Zhao, Y., Huang, W., Hu, W., Gu, J., Chen, C., ... & Wang, Z. (2014). Sperm motility inversely correlates with seminal leptin levels in idiopathic asthenozoospermia. *International journal of clinical and experimental medicine*, 7(10), 3550.
- [35] Rosen, E. D., & Spiegelman, B. M. (2006). Adipocytes as regulators of energy D., & Spiegelman, B. M. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature*, 444(7121), 847-853.
- [36] Holdstock, C., Engström, B. E., Öhrvall, M., Lind, L., Sundbom, M., & Karlsson, F. A. (2003). Ghrelin and adipose tissue regulatory peptides: effect of gastric bypass surgery in obese humans. *The Journal of Clinical Endocrinology & Metabolism*, 88(7), 3177-3183.