

Original Research Article

Association of The T45G Polymorphism of Adiponectin Gene with Polycystic Ovary Syndrome in Women of Babylon Province/ Iraq

Hawraa Sabah Al-Musawi
College of Science, University of Babylon, Hilla, IRAQ

E-mail:lahzg.1983@gmail.com

Accepted 9 December, 2015

Abstract

Polycystic Ovarian syndrome (PCOS) is being the most frequent cause of anovulatory infertility, Adiponectin is the most abundant adipocytokine and may play a role in the regulation of insulin sensitivity and IR in PCOS and count for 0.01% or 3–30 µg/ml of total plasma protein. The adiponectin gene contains 3 exons spans 16 kb on chromosome 3q27. The aim of the present study was to evaluate the genetic influence of the adiponectin gene polymorphisms in the development of PCOS among women of Babylon Province/ Iraq. sixty three women were studied, and were classified into two groups of: first group consists of 32 women infected with polycystic ovaries syndrome, the second consists of 31 healthy women to detect the presence of T45G polymorphism within the gene. From all subjects a whole-blood sample was taken and was used for isolation of peripheral blood leukocytes. The adiponectin T45G polymorphism, located in exon 2, was genotyped by amplification of genomic DNA. The present study included study of the relationship of this gene with PCOS women in reproductive age, A statistically significant difference was observed in the frequency of TT, TG and GG genotypes between women with PCOS and controls.

Key words: PCOS, T45G polymorphism, Adiponectin gene

الخلاصة

تعد متلازمة تكيس المبايض (PCOS) Polycystic Ovarian syndrome السبب الأكثر شيوعاً لغياب الإباضة في العقيمات، اديبونيكتين هو adipocytokine الأكثر شيوعاً ويمكن أن يلعب دوراً في تنظيم حساسية الأنسولين و مقاومة الانسولين في PCOS ويكون نسبة ٠.٠١٪ أو ٣-٣٠ ميكروغرام / مل من البروتين الكلي في البلازما يحتوي جين اديبونيكتين على ثلاثة مناطق للتشفير (3 exons) يمتد ١٦ كيلو بايت 16 kb على الكروموسوم 3q27. الهدف من هذه الدراسة هو تقييم التأثير الوراثي لتعدد الأشكال في جين اديبونيكتين في تطور متلازمة تكيس المبايض بين النساء في محافظة بابل/العراق. تمت دراسة ثلاث وستين امرأة، وصنفت إلى مجموعتين: تتكون المجموعة الأولى من ٣٢ امرأة مصابة بمتلازمة تكيس المبايض، وتتكون المجموعة الثانية من ٣١ امرأة يتمتعن بصحة جيدة للكشف عن وجود تعدد الأشكال خلال هذا الجين. وتم أخذ عينة دم كامل من جميع عينات الدراسة ولعزل الكريات البيضاء في الدم المحيطي. تم ايجاد تعدد الأشكال جين T45G ، والتي تقع في منطقة التشفير (exon 2)، من خلال تضخيم الحمض النووي الجيني DNA. وشملت هذه الدراسة دراسة العلاقة بين هذا الجين مع النساء PCOS في سن الإنجاب، ولوحظ وجود فروق ذات دلالة إحصائية في الأنماط الجينية TT، TG و GG بين النساء المصابات بمتلازمة تكيس المبايض ومجموعة السيطرة.

الكلمات المفتاحية: مبيض متعدد الاكياس ، T45Gتعدد الاشكال، اديبونيكتين جين.

Introduction

Polycystic Ovarian syndrome (PCOS) is one of important Hormones' distribution disease which affected fertile women with an

estimated prevalence of 4–8 % [1]. The ESHRE/ASRM consensus conference held in Rotterdam in 2003 defined the syndrome as having two of the following three conditions diagnosed as PCOS:

oligo-ovulation; clinical or biochemical evidence of androgen excess; and multicystic ovaries [2]. The clinical symptoms of (PCOS) characterized by hirsutism; Irregular cycle; anovulation and obesity especially in abdominal region as a result of increasing of male hormones (androgen) [3]. In addition to its reproductive features, PCOS also has numerous metabolic consequences, including increased risk of obesity [4], insulin resistance (IR) [5], type 2 diabetes mellitus (T2DM) [6] and premature arteriosclerosis [7].

The adiponectin was identified in 1995 by [8]. It is the most abundant adipocytokine and accounts for 0.01% of total plasma protein [9], studies [9,10] revealed that levels of adiponectin are reduced in obese and type 2 diabetes in comparison with normal individuals, the women with PCOS are also found to have lower adiponectin levels than the normal controls. A number of studies also suggest that serum adiponectin level correlates on the contrary with insulin resistance [11]; low levels of adiponectin are consistently associated with a higher risk of type 2 diabetes [12].

Adiponectin has been shown to have antiatherogenic effects [13,14].

The adiponectin gene composed of three exons and two introns spanning a 17-kb region, and has been located on chromosome 3q27 [8,15,16], this gene coding for a 30-kDa protein that consists of an N terminal collagenous domain and a C-terminal globular domain [17]; One common and two rare genetic polymorphisms in the adiponectin gene have been identified in non-diabetic populations [18,15]. The silent T/G polymorphism in exon 2 of the human adiponectin gene (T45G!Gly15Gly) could somehow affect plasma adiponectin levels [19,20].

Despite the numerous reports studying the association between adiponectin gene polymorphisms and insulin resistance or obesity, until now, few studies have examined adiponectin gene

polymorphisms in women with PCOS. In this study, we investigated the possible association of the T45G adiponectin gene polymorphism with PCOS in Iraqi Babylon patients.

Material and Method

Thirty-two PCOS patients and thirty-one healthy controls in reproductive age were collected from Babylon hospital for the period from September 2014 to March 2015, the sample was collected and stored in test tube with EDTA and freezing at (-86°C) for DNA extraction. Genomic DNA was isolated from peripheral blood leukocytes of women with PCOS and the controls using ReliamprepTM blood gDNA Miniprep System (Promega). The adiponectin T45G polymorphism, located in exon 2, was genotyped by amplification of genomic DNA using the following primers: F50-GAA TGAGACTCTGCTGAGATGG and R50-

TATCATGTGAGGAGTGCTTGGATG. [21]. PCR products were obtained using 25ml reactions of Go Tag Green Master Mix (Promega) using 3 ml of template DNA and 1 ml of (10PM) forward and reverse primer by Verti 96 thermo cycler (Applied Biosystem). The amplification conditions were as follows: 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 30°C and 45 s at 72°C, and ending with a single 10 min extension step at 72°C. The resulting fragment was 372 bp in length. The polymorphism was typed with enzyme SmaI (Bio Labs Inc., New England). Digestion of the G allele produced two fragments with lengths 216 and 156 bp. The digestion products were resolved after electrophoresis in 2% agarose gel containing ethidium bromide (Figure 1).

Statistical Analyses

Genotype and allele distribution was compared between cases (women with PCOS) and controls using Pearson's χ^2 test [22].

Results and Discussion

Three pattern of genotype were obtained in current study: TT, TG and GG, The results revealed as shown in Table (1) The percentage of genotype(TT) in PCOS women were (59.4 %) While in control group (74.2 %), the percentage rate of

genotype (TG) were(31.3%),(25.8%) in patients and control group respectively, while genotype(GG) absent in the control group and were represented in the group of PCOS women with percentage (9.3 %).

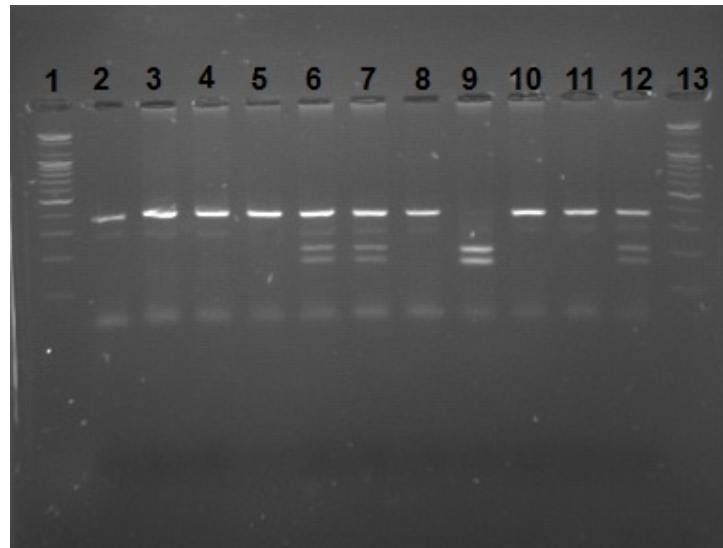


Figure 1 : Gel electrophoresis of PCR products of adiponectin gene treated with SmaI FOR 3Hours at 25° C IN 2% agarose gel at 100 volt/cm² for 30 min. then 50 volt for 90 min. visualized by U.V. Lane 1,13 DNA ladder (100-1000), Lane (2-5,8,10,11) samples with TT genotype Lane (6,7,12) samples with TG genotype Lane (9) sample with GG genotype

Table 1 : Genotyping in the gene with polycystic ovary syndrome (PCOS) women

Genotyping	(PCOS)		CONTROL	
	NO.	%	NO.	%
TT	19	59.4	23	74.2
TG	10	31.3	8	25.8
GG	3	9.3	0	0
TOTAL	32		31	

Although no difference was observed when X2-test was performed on the distributions of the three genotypes separately, a statistically significant difference

($\chi^2 = 108$, $P < 0.05$) was observed in the frequency of TT, TG and GG genotypes between women with PCOS. figure (2).

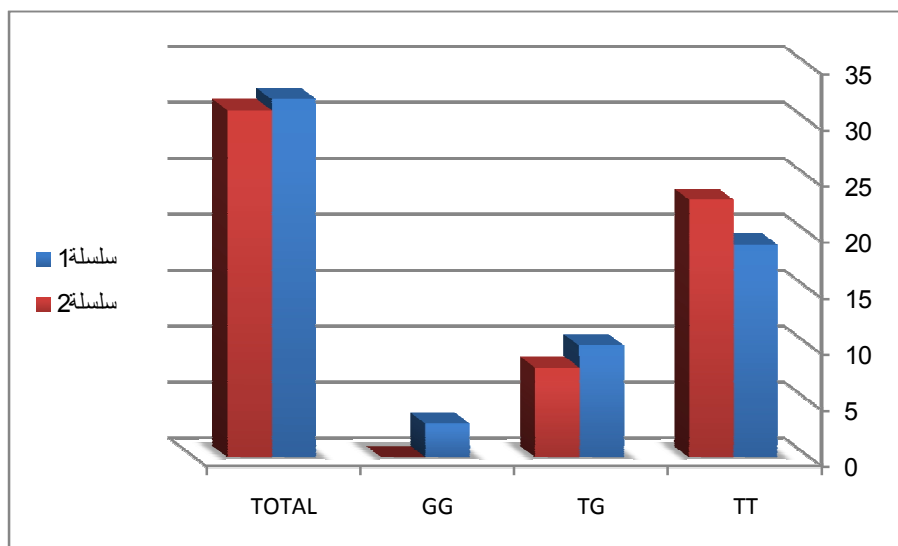


Figure 2: Frequency of genotype TT, TG & GG in (PCOS) women and control, show significant difference under ($P < 0.05$)

It's interesting that this study was first recorded presence of statically significant difference in the frequency of genotype (TT), (TG) and (GG) for T45G polymorphism of adiponectin gene in Iraqi PCOS patients compared with control, also The study report for a first time the lack of (GG) genotype of the control group and its presence in Iraqi patients with PCOS. The disappearance of GG genotype from the control group may reflect the importance of this gene in the incidence of the disease since the meeting of the two mutant alleles (G) in the same woman leads to the disease in addition to increasing the percentage genotype (TT) in the control group compared to women with PCOS.

Our findings conceptual agreement with a previous study on a different ethnic group (Greek women) with PCOS, which found that the TG and GG genotypes of the 45TG polymorphism were more frequent in women with PCOS than in controls, and these particular genotypes were associated with higher 4-androstenedione concentrations [21]. The current study also corresponds with another study

Conducted on Han Chinese women were also found a significant difference between the genotypes of this polymorphism and showed decrease the presence of TG and GG genotype in the control group compared with PCOS women group and this consistent with present study, so it seems that these genotypes are a risk factor for PCOS, because it increases the bioavailability of androgens in women with PCOS [23]. While the results of the current study did not conform to a Greek study [24] conducted on women with PCOS which found no differences in the distributions of T45G polymorphism between women with PCOS, and control. And they also refer to the emergence of GG genotype in controls group and absence it in patients with PCOS. They suggest these genomic variants may influence production of adiponectin and the metabolic variables related to insulin resistance/metabolic syndrome in patients with PCOS. On the other hand, an Italian study, shows a significant association between this polymorphism, obesity and features of insulin resistance [25]. Several studies

have reported an association between This highly prevalent polymorphism and the risk of obesity, insulin resistance, DM2 and high levels of low-density lipoprotein cholesterol [20,26, 27]. While other studies have not documented any association for particular locus with obesity or DM2[28,29,30].

The silent T/G polymorphism in exon 2 of the human adiponectin gene (T45G ' Gly15Gly) can affect one way or another plasma adiponectin levels [19,20].

As well as [25] shows that this polymorphisms might be in linkage disequilibrium, and the G/G haplotype has been strongly associated with the metabolic disturbances of PCOS, as well as lower plasma adiponectin concentration.

Therefore, although the number of women homozygous for the G allele in our study was relatively small to draw firm conclusions, we postulate that there is a complex relationship, possibly a negative feedback loop, between adiponectin and the hypothalamic–pituitary–gonadal axis, specifically steroid synthesis or action. Indeed, in vitro studies have shown that both glucocorticoids and androgens [31] down-regulate the expression of adiponectin, and there is substantial evidence suggestive of a complex interaction between this hormone and gonadal function [32].

In conclusion, the number of genomic variants associated with PCOS is growing rapidly, suggesting that PCOS may result from the interaction of multiple genomic variants and environmental factors such as obesity and a sedentary lifestyle although the physiological significance of such a relationship remains obscure at present. Since the T45G polymorphism is a synonymous mutation, the exact molecular mechanisms responsible for the biological effects of this variation are not known at present. It is plausible that this polymorphism is in linkage disequilibrium with some other functional genetic alterations. More data

are needed to specify the systemic and local function of the newly identified 'adipocytokines' in the pathophysiology of PCOS.

References

1. Azziz, R., Wood, K.S., Reyna, R., Key, T.S., Knochenhauer, E.S. and Yildiz, B.O. (2004). The prevalence and features of the polycystic ovary syndrome in an unselected population, *J. of Clinical Endocrinology and Metabolism*. Vol. 89: (6). pp. 2745-2749
2. Unluturk, U., Harmanci, A., Kocaefer, C. and Yildiz, B.O. (2007). The genetic basis of the polycystic ovary syndrome: A literature review including discussion of *PPAR*. Hindawi Publishing Corporation. Vol. 207. Pp 1-23.
3. AL-Jabery K M S. (2010). The relationship between some clinical symptoms and Some physiological and hormonal and biochemical blood standards in women affected with Polycystic ovary syndrome. PhD. College of Science. Kufa university.
4. Hoeger, K. (2001). Obesity and weight loss in polycystic ovary syndrome. *Obstet Gynecol. Clin. North Am.*; (28) :85–97. [PubMed: 11293006]
5. Cotrozzi, G.; Matteini, M.; Relli, P. and Lazzari, T. (1983). Hyperinsulinism and insulin resistance in polycystic ovarian syndrome: a verification using oral glucose, I.V. Glucose and tolbutamide. *Acta Diabetol. Lat.*; (20):135–42. [PubMed: 6224386]
6. Legro, R.S.; Kusanman, A.R.; Dodson, W.C.; Dunaif, A. (1999). Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin. Endocrinol. Metab.*; (84):165–9. [PubMed: 9920077]
7. Talbott, E.O.; Guzick, D.S.; Sutton-Tyrrell, K.; McHugh-Pemu, K.P.; Zborowski, J.V.; Rensberg, K.E. and Kuller, L.H. (2000). Evidence for

- association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler. Thromb. Vasc. Biol.*; (20):2414–21. [PubMed: 11073846]
8. Scherer, P.E.; Williams, S.; Fogliano, M.; Baldini, G. and Lodish, H.F. (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *J. Biol. Chem.* (270): 26746–26749.
 9. Arita, Y.; Kihara, S.; Ouchi, N.; Takahashi, M.; Maeda, K.; Miyagawa, J.; Hotta, K.; Shimomura, I.; Nakamura, T.; Miyaoka, K.; Kuriyama, H.; Nishida, M.; Yamashita, S.; Okubo, K.; Matsubara, K.; Muraguchi, M.; Ohmoto, Y.; Funahashi, T. and Matsuzawa, Y. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications*: 257 79–83.
 10. Ardawi, M.S. and Rouzi, A.A. (2005). Plasma adiponectin and insulin resistance in women with polycystic ovary syndrome. *Fertility and Sterility*; (83): 1708–1716.
 11. Goldstein, B.J.; Scalia, R.G. and Ma, X.L. (2009). Protective vascular and myocardial effects of adiponectin. *Nat. Clin. Pract. Cardiovasc. Med*, 6(1): 27–35.
 12. Li, S.; Shin, H.J.; Ding, E.L. and van Dam, R.M. (2009). Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA*, 302: 179–188.
 13. Okamoto, Y.; Arita, Y.; Nishida, M.; Muragushi, M.; Ouchi, N.; Takahashi, M.; Igura, T.; Inui, Y.; Kihara, S. and Nakamura, T. (2000) An adipocyte derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm. Metab. Res.*; (32):47–50.
 14. Ouchi, N.; Kihara, S.; Arita, Y.; Nishida, M.; Matsuyama, A.; Okamoto, Y.; Ishigami, M.; Kuriyama, H.; Kishida, K. and Nishizawa, H. (2001). Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* (103):1057–1063.
 15. Takahashi, M.; Arita, Y.; Yamagata, K.; Matsukawa, Y.; Okutomi, K.; Horie, M.; Himomura, I.; Hotta, K.; Kuriyama, H. and Kihara, S. (2000). Genomic structure and mutations in adipose-specific gene, adiponectin. *Int. J. Obes. Relat. Metab. Disord.*; (24):861–868.
 16. Francke, S.; Manraj, M.; Lacquemant, C.; Lecoeur, C.; Lepretre, F.; Passa, P.; Hebe, A.; Corset, L.; Yan, S.L.; Lahmidi, S.; Jankee, S.; Gunness, T.K.; Ramjuttun, U.S.; Balgobin, V.; Dina, C. and Froguel, P. A. (2001). Genome-wide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. *Hum. Mol. Gen.*; (10):2751–2765.
 17. Pajvani, U.B.; Du, X.; Combs, T.P.; Berg, A.H.; Rajala, M.W.; Schulthess, T.; Engel, J.; Brownlee, M. and Scherer, P.E. (2003). Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin: Implications for metabolic regulation and bioactivity. *J. Biol. Chem.*, 278(11): 9073–9085.
 18. Schaffler, A.; Barth, N.; Palitzsch, K.D.; Drobnik, W.; Scholmerich, J. and Schmitz, G. (2000). Mutation analysis of the human adipocyte-specific apM-1 gene. *Eur. J. Clin. Invest.*, (30):879–887.
 19. Yang, W.S.; Hsiung, C.A.; Ho, L.T.; Chen, Y.T.; He, C.T.; Curb, J.D.; Grove, J.; Quertermous, T.; Chen, Y.D.I.; Kuo, S.S. (2003a). Genetic epistasis of adiponectin and PPAR γ 2 genotypes in modulation of insulin sensitivity: a family-based association study. *Diabetologia* (46):977–983.
 20. Yang, W.S.; Tsou, P.L.; Lee, W.J.; Tseng, D.L.; Chen, C.L.; Peng, C.C.; Lee, K.C.; Chen, M.J.; Huang, C.J. and Tai, T.Y. (2003b). Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. *J. Mol. Med.*, (81):428–434.
 21. Panidis, D.; Kourtis, A.; Asterios Kuku, Vitis, A.; Dimitrios Farma

- kiotis, D.;Xita,N.; Georgiou, I. and Tsatsoulis,A.(2004). Association of the T45G polymorphism in exon 2 of the adiponectin gene with polycystic ovary syndrome: role of D4-androstenedione . Hum . Repro. Vol.19, No.8 pp. 1728–1733.
22. Niazi, A.D. (2004). Statistical analysis in Medical Research. 2nd ed. Coll.of Med., Nahrain Univ. Baghdad. P: 73-98.
23. Zhang,N.; Shi, Y.; Hao,C.; Gu,H.F.; Li,Y.; Zhao, Y.; Wang,L.and Chen,Z.(2008). Association of C45G15G(T/G) and C276(G/T) polymorphisms in the ADIPOQ gene with polycystic ovary syndrome among Han Chinese women. Europ. J. Endocrin., (158):255–260.
- 24.Xita,N.; Georgiou,I.; Chatzikyriakidou,A.;Vounatsou,M.; Papassotiriou,G.; Papassotiriou.I.and Tsatsoulis,A. (2005). Effect of Adiponectin Gene Polymorphisms on Circulating Adiponectin and Insulin Resistance Indexes in Women with Polycystic Ovary Syndrome . Clin. Chem.(51):2416–423.
25. Menzaghi, C.; Ercolino, T.; Di Paola, R.; Berg, A.H.; Warram, J.H. and Scherer,P.E. (2002).A haplotype at the adiponectin locus is associated withobesity and other features of the insulin resistance syndrome.Diabetes, (51):2306–12.
26. Stumvoll,M.; Tschritter, O.; Fritsche, A.; Staiger, H.; Renn, W.; Weisser, M.;Machicao, F. and Haaring, H. (2002).Association of the T-G polymorphism in adiponectin gene (exon 2) with obesity and insulin sensitivity: interactionwith family history of type 2 diabetes. Diabetes,(51): 37–41.
27. Ukkola, O.; Ravussin, E.; Jacobson, P.; Sjostrom, L. and Bouchard, C.(2003).Mutations in the adiponectin gene in lean and obese subjects from theSwedish obese subjects cohort. Metabolism,(52): 881–884.
28. Schaffler, A.; Barth, N.; Palitzsch, K.D.; Drobnik, W.; Scholmerich, J. and Schmitz,G. (2000). Mutation analysis of the human adipocyte-specific apM-1 gene.Eur. J. Clin. Invest.,(30): 879–887.
29. Zietz, B.; Barth, N.; Scholmerich,J.; Schmitz, G. and Schaffler, A. (2001).Gly15Gly polymorphism within the human adipocyte specific apM-1 gene but not Tyr111His polymorphism is associated with higher levels of cholesterol and LDL-cholesterol in caucasian patients with type 2 diabetes. Exp. Clin. Endocrinol. Diabetes, (109):320–325.
30. Filippi, E.; Sentinelli, F.; Trishitta, V.; Romeo, S.; Area, M.; Leonetti, F.; Di Mario, U.andBaroni,M.G. (2004). Association of the human adiponectin gene andinsulin resistance. Eur .J. Hum. Genet. in press.
31. Nishizawa, H.; Shimomura, I.; Kishida, K.; Maeda, A.; Kuriyama, H.; Nagaretani,H.; Matsuda, M.; Kondo, H.;Furuyama, N. and Kihara,S. (2002).Androgensdecrease plasma adiponectin, an insulin-sensitizing adipocyte derived protein.Diabetes. (51): 2734–2741.
32. Combs,T.P.; Berg, A.H.; Rajala, M.W.; Klebanov, S.; Iyengar, P.; Jimenez-Chillaron, J.C.; Patti, M.E.; Klein, S.L.; Weinstein, R.S. and Scherer, P.E. (2003). Sexual differentiation, pregnancy, calorie restriction and aging affect the adipocyte-specific protein adiponectin. Diabetes.(52): 268–276.