ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 75–79

THE JOURNAL OF Nutritional Biochemistry

Elevated plasma total homocysteine levels in hyperinsulinemic obese subjects

Víctor Sánchez-Margalet[a,*], Miguel Valle[b], Francisco J. Ruz[b], Félix Gascón[b], Joaquín Mateo[a], Raimundo Goberna[a]

[a]*Department of Clinical Biochemistry, Investigation Unit, Virgen Macarena University Hospital, Seville, Spain*
[b]*Hospital Valle de los Pedroches, Córdoba, Spain*

Received 4 January 2001; received in revised form 2 July 2001; accepted 11 September 2001

Abstract

Homocysteine has been associated with the oxidative stress in the pathogenesis of atherosclerosis. Oxidative stress caused by triglycerides and free fatty acids is known to cause insulin resistance and hyperinsulinemia. On the other hand, insulin resistance may increase homocysteine levels. Since obesity is associated with insulin resistance and hyperinsulinemia, we aimed to study the possible association of homocysteine with hyperinsulinemia in obese subjects. 20 obese male subjects (body mass index >29), aged 33–55 (mean 45 years old) were studied. A fasting blood sample was obtained for the study and the subjects undertook an oral glucose tolerance test with samples taken at 1 and 2 h after glucose. Subjects were divided in two groups according to the fasting insulin levels, < 9 μU/ml or normoinsulinemic (group 1) and >9 μU/ml or hyperinsulinemic (group 2). Glucose, insulin, homocysteine, folate, $B_{12,}$ total cholesterol, HDL-cholesterol and triglycerides levels were determined in fasting blood samples. In oral glucose tolerance test, glucose, insulin and homocysteine levels were measured. Hyperinsulinemic obese subjects (group 2) had higher levels of insulin and glucose at 1 h and 2 h postglucose, compared with group 1. Fasting total homocysteine and triglyceride levels were also increased in this group, whereas folate and B_{12} levels were similar in both groups. Fasting homocysteine significantly correlated with fasting insulin ($r = 0.6$, $p < 0.01$). Homocysteine levels slightly but significantly decreased after glucose loading in normoinsulinemic but not in hyperinsulinemic obese subjects. These results show that higher homocysteine levels are observed in the hyperinsulinemic obese subjects and suggest that homocysteine could play a role in the higher risk of cardiovascular disease in obesity.

Keywords: Obesity; Homocysteine; Vitamines; Hyperinsulinemia; Triglycerides; Oral glucose tolerance test (OGTT)

1. Introduction

Hyperhomocysteinemia is a well established independent risk factor for atherosclerotic and thromboembolic vascular disease [1–3]. Hereditary enzymatic deficiencies and nutritional deficiencies of folate, pyridoxine or cobalamin (B_{12}), as well as chronic renal failure are associated with elevated blood homocysteine and accelerated atherosclerosis [1–3]. The mechanism of homocysteine angiotoxicity seems to involve the nitric oxide system by inducing oxidant stress [4–6]. Oxidative stress has been suggested to cause insulin resistance and may be its possible link with atherosclerosis [7,8]. Thus, oxidant stress reduces insulin responsiveness in vitro by interrupting insulin signaling [9,10], and may impair insulin mediated glucose uptake in healthy subjects and those with noninsulin-dependent diabetes mellitus (NIDDM) [11–13]. In fact, there seems to be a relationship between oxidative stress and metabolic control in NIDDM [14]. Moreover, the administration of reduced glutathion has been shown to increase glucose uptake in both healthy and patients with NIDDM [15].

Hyperhomocysteinemia has been found in patients with NIDDM and IDDM, with an association with premature atherosclerosis [16–18]. Furthermore, insulin resistance has been recently found to be associated with elevated plasma total homocysteine levels in healthy non-obese subjects [19]. In fact, plasma homocysteine concentrations seem to be regulated by acute hyperinsulinemia in nondiabetic but not in type 2 diabetic subjects [20], suggesting that insulin resistance may contribute to the development of hyperhomocysteinemia and therefore have implications of premature vascular disease.

* Corresponding author. Tel.: +34–95 500 8107; fax: +34–95 500 8105.

E-mail address: Vsanchez@cica.es (V. Sanchez-Margalet).

0955-2863/02/$ – see front matter
PII: PII S0955-2863(01)00197-8

Table 1
Clinical characteristics of the subjects. Values are expressed as means ± SEM. Group 1, subjects with fasting insulin levels < 9 μU/ml. Group 2, subjects with fasting insulin levels > 9 μU/ml

	Group 1 (n = 8)	Group 2 (n = 12)
Age (years)	45 ± 2.2	46 ± 3.1
BMI (kg/m^2)	32 ± 1.5	34 ± 1.6
Waist/hip ratio	1.01 ± 0.01	1.02 ± 0.01
Fasting glucose (mg/dl)	101 ± 2.5	102 ± 3.5
Fasting insulin (μU/ml)	6 ± 0.6	16 ± 2.2*

* $p < 0.01$ vs. group 1.

In this context, we have studied total homocysteine levels in obese subjects to find out a possible association with the hyperinsulinemia that is usually present in this syndrome.

2. Materials and methods

2.1. Subjects

Obese subjects (body mass index >29), non-diabetic (basal glucose <125 mg/ml) and non-hypertensive (systolic pressure <140 mmHg, diastolic pressure <90 mmHg) from the Hospital de los Pedroches clinic were studied. They were chosen randomly from the general practice clinic population of the region (Northern area of Cordoba Province). Clinical characteristics of the subjects are shown in Table 1. They were all male and caucasians and with no major pathological problems. They were divided into two groups, according to the basal insulin levels: Group 1, normoinsulinemic (< 9 μU/ml), and Group 2, hyperinsulinemic (> 9 μU/ml). The rationale for this insulin concentration limit relies on the epidemiological and prospective data about the increased risk for cardiovascular disease for insulin above these levels [21,22]. To further assess the insulin resistance state in these subjects, they undertook an oral glucose tolerance test, receiving 75 g glucose [23]. Plasma glucose, insulin and homocysteine were measured at 0, 60 and 120 min.

2.2. Biochemical and hormone determinations

Blood samples were taken in EDTA-k_3 tubes (Vacutainer, Becton Dickinson), immediately centrifuged at 4°C and plasma separated and stored at -20C. Glucose levels were determined by the glucose oxidase method [24]. Insulin, homocysteine, folate and B_{12} levels were measured by an IMX-System autoanalyser (Abbot Cientifica, Madrid)). Triglycerides and total and high-density lipoprotein-cholesterol were analised by enzymatic colorimetric test and precipitation technique (phosphotungstate-magnesium chloride; kit from Boehringer-Mannheim GmbH, Barcelona, Spain).

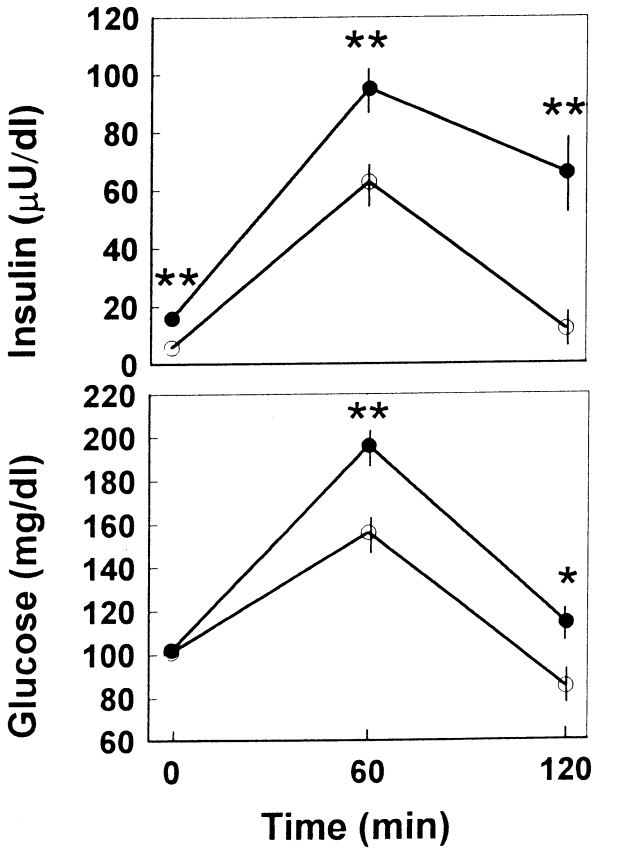

Fig. 1. Oral glucose tolerance test in obese subjects. Insulin (upper panel) and glucose (lower panel) were determined before and 60 min and 120 min after oral glucose loading (75 g). Open circles refer to normoinsulinemic obese subjects (group 1). Closed circles refer to hyperinsulinemic obese subjects (group 2). * $p < 0.05$; ** $p < 0.01$.

2.3. Statistical analysis

Values are expressed as means ± S.E.M. Data were analyzed by analysis of variance for multiple comparison, and post test (Bonferroni) was used to test the degree of significance of the differences between groups.

3. Results

3.1. Oral glucose tolerance test

Normoinsulinemic (group 1) and hyperinsulinemic (group 2) obese subjects undertook an oral glucose tolerance test to better assess their insulin resistant state. As shown in Fig. 1, group 2 had significantly increased post-glucose levels (at 60 and 120 min) compared with group 1, even though glucose values did not reach levels high enough to consider these subjects as glucose intolerant. These higher glucose levels were observed in group 2 in spite of the fact that insulin levels raised significantly higher than those of group 1 (at 60 and 120 min), revealing the

Table 2
Homocysteine, folate, B_{12} and lipid levels in hyper- and normoinsulinemic obese subjects

	Group 1	Group 2
Homocysteine (μmol/l)	7.1 ± 0.7	12.4 ± 0.5*
Folate (ng/ml)	6.3 ± 0.6	6.0 ± 0.4
B_{12} (pg/ml)	670 ± 60	656 ± 80
Triglycerides (mg/dl)	70 ± 7	145 ± 19**
Total cholesterol (mg/dl)	214 ± 8	215 ± 12
HDL cholesterol (mg/dl)	46 ± 3	48 ± 4

* $p < 0.05$; ** $p < 0.01$ versus group 1.

insulin resistance state of this group of hyperinsulinemic obese subjects. This group of insulin resistant obese subjects turned out to be the 60% (12 out of 20) of the subjects. The normoinsulinemic obese subjects were the 40% and were found to have normal insulin response to oral glucose even though they had no significant differences in body mass index and waist/hip ratio with group 2 (Table 1).

3.2. Homocysteine, folate, B_{12} and lipid levels

As shown in Table 2, total homocysteine levels in group 2 (hyperinsulinemic obese subjects) were found significantly increased compared to those in group 1 (12.4 versus 7.1 μmol/L). However, folate and B_{12} levels were similar in both groups and within normal ranges (Table 2). The normal range for the method employed is 3.1–12.4 ng/ml for folate levels, and 223-1132 pg/ml for $B_{12.}$ No differences in total cholesterol or HDL cholesterol were found between both groups. Cholesterol values were in the normal range. However, triglycerides were significantly increased in insulin resistant obese subjects (group 2) (145 versus 77 mg/dl), although they did not reach pathological values.

In normoinsulinemic obese subjects (group 1), total homocysteine levels slightly but significantly decreased in response to glucose loading (Fig. 2) after 1 h and these levels were maintained after 2h post-glucose. However, in hyperinsulinemic obese subjects (group 2) total homocysteine levels did not change upon glucose challenge.

3.3. Correlation of fasting homocysteine with fasting insulin levels

Since homocysteine levels were increased in the hyperinsulinemic group, we assessed the correlation between both parameters. As shown in Fig. 3, homocysteine plasma levels correlated with those of insulin in fasting conditions ($r = 0.6$, $p < 0.01$). Even though triglicerides levels were also increased in hyperinsulinemic obese subjects, there was no significant correlation between plasma triglyceride and homocysteine levels ($r = 0.1$, $p < 0.4$) or insulin (0.1, $p < 0.5$).

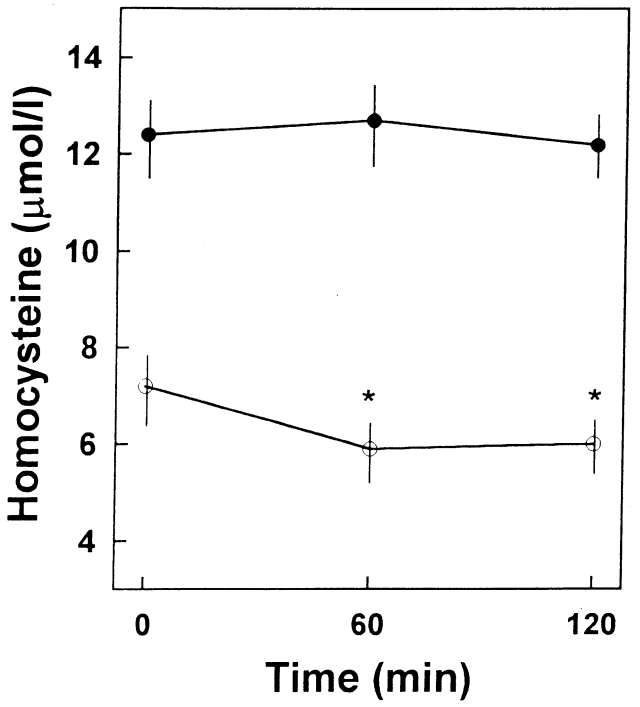

Fig. 2. Homocysteine levels in response to the oral glucose loading. Open circles refer to normoinsulinemic obese subjects (group 1). Closed circles refer to hyperinsulinemic obese subjects (group 2). * $p < 0.05$

4. Discussion

Obesity, specially visceral obesity, is a major risk factor for NIDDM and cardiovascular complications [25]. Hyperinsulinemia and hyperhomocysteinemia are now other well accepted risk factors for atherosclerosis [1,25]. However, we do not know yet the exact relationship between these risks factors. We have found that increased total homocysteine levels are present in obese subjects with hyperinsulinemia (> 9 μU/ml) compared with obese subjects with normal insulin levels (< 9 μU/ml). A possible role of nutritional deficiencies of folate and B_{12} should be ruled out, since similar plasma levels of these vitamines were found in both groups and they were always above minimal normal levels. The association between insulin resistance and homocysteine has recently been proposed in healthy, non-obese subjects [19]. A common feature in insulin resistant subjects is the hyperlipidemia with increased levels of triglycerides and very low density lipoproteins (VLDL) [26]. In fact, significant differences were found in triglyceride levels between the two groups of obese subjects. Triglycerides are known to impair insulin action through an increase in oxidative stress [27,28], and therefore they may somehow account for the insulin resistance observed in group 2. Since homocysteine seems to induce oxidative stress [4–6], the increase in homocysteine levels observed in insulin resistant obese subjects (group 2) may contribute to the pathophysiology of these obese subjects. Nevertheless, the differences in homocysteine levels are no so dra-

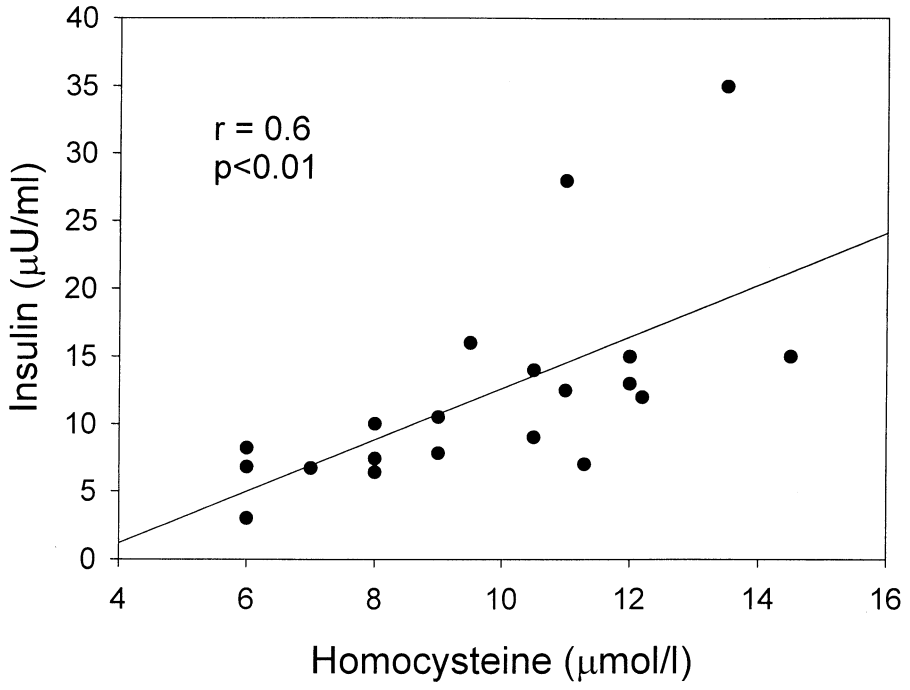

Fig. 3. Correlation of homocysteine with insulin plasma levels in obese subjects.

matic to think that they are responsible for the insulin resistance. On the contrary, high homocysteine levels may be the consequence rather than the cause of the insulin resistance. In fact, insulin has been shown to negatively regulate homocysteine levels [20]. Thus, a lack of effect of insulin may produce this elevation in homocysteine levels. In this line, we found that homocysteine levels decreased after glucose loading in normoinsulinemic subjects, whereas they did not change in hyperinsulinemic group. Moreover, we have found a significant positive correlation between fasting insulin and homocysteine plasma levels. These findings are in line with a role of insulin resistance in the increase in homocysteine levels. Moreover, the oxidative stress present in insulin resistant subjects such as type 2 diabetics [14] may worsen the angiotoxicity of homocysteine, increasing the risk for atherosclerosis. On the other hand the increase in homocysteine levels may also worsen the insulin resistance through the oxidative stress, closing a possible dangerous vicious circle. In this context, we have recently found that homocysteine induces insulin resistance in vitro at high concentrations (50 μM) by inhibiting insulin signaling, and this effect may be mediated by oxidative stress, since it can be prevented by glutathione [29].

In any case the present study does not permit conclusions about whether elevation of plasma homocysteine is the cause or result of insulin resistance. Moreover, other possible sources of elevated homocysteinemia, such as genetic variation of methylenetetrahydrofolate reductase activity or functional vitamin B6 deficiency [2] have not been assessed in the present study.

The increase in homocysteine levels may not be a general complication of the insulin resistance syndrome, since healthy volunteers with altered insulin-mediated glucose disposal have recently been shown to have no increased homocysteine levels [30]. Nevertheless, in hyperinsulinemic obese subjects there seems to be an association with increased homocysteine levels, which may contribute to the development of cardiovascular complications which are so common in obesity.

Acknowledgments

This work was supported by the Virgen Macarena Hospital, Servicio Andaluz de Salud, Andalucía, Spain. We are greatful to Abbot Cientifica (Madrid) for generously providing the reagents to measure folate and B_{12} levels.

References

[1] K.S. McCully, Homocysteine and vascular disease, Nature Med. 2 (1996) 386–389.
[2] S.S. Kang, P.W. Wong, Genetic and nongenetic factors for moderate hyperhomocys(e)inemia, Atherosclerosis 119 (1996) 135–138.
[3] C.J. Boushey, S.A. Beresford, G.S. Omenn, A.G. Motulsky, A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes, J. Am. Med. Assoc. 274 (1995) 1049–1057.
[4] A. Tawakol, T. Omland, M. Gerhard, J.T. Wu, M.A. Creager, Hyperhomocyst(e)inemia is associated with inpaired endothelium-dependent vasodilation in humans, Circulation 95 (1997) 1119–1121.
[5] J. Loscalzo, The oxidant stress of hyperhomocyst(e)inemia, J. Clin. Invest. 98 (1996) 5–7.

[6] G. Blundell, B.G. Jones, F.A. Rose, N. Tudball, Homocysteine mediated endothelial cell toxicity and its amelioration, Atherosclerosis 122 (1996) 163–172.
[7] A. Ceriello, M. Pirisi, Is oxidative stress the missing link between insulin resistance and atherosclerosis? Diabetologia 38 (1995) 1484–1485.
[8] I. Wittmann, J. Nagy, Are insulin resistance and atherosclerosis the consequences of oxidative stress? Diabetologia 39 (1996) 1002–1003.
[9] A. Rudich, N. Kozlovsky, R. Potashnik, N. Bashan, Oxidant stress reduces insulin responsiveness in 3T3–L1 adipocytes, Am. J. Physiol. 272 (1997) E935–E940.
[10] A. Tirosh, R. Potashnik, N. Bashan, A. Rudich, Oxidative stress disrupts insulin-induced redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3–L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and glut4 translocation, J. Biol. Chem. 274 (1999) 10595–10602.
[11] G. Paolisso, A. D'Amore, G. Di Maro, D. Galzerano, P. Tesauro, M. Varricchio, F. D'Onofrio, Evidence for a relationship between free radicals and insulin action in the elderly, Metabolism 42 (1993) 659–663.
[12] G. Paolisso, D. Giuliano, Oxidative stress and insulin action: is there a relationship? Diabetologia 39 (1996) 357–363.
[13] G. Paolisso, A. D'Amore, C. Volpe, V. Balbi, F. Saccomanno, D. Galzerano, et al, Evidence for a relationship between oxidative stress and insulin action in non-insulin dependent diabetic patients, Metabolism 43 (1994) 1426–1429.
[14] J. Nourooz-Zadeh, A. Rahimi, J. Tajaddini-Sarmadi, H. Tritschler, P. Rosen, B. Halliwell, D.J. Betteridge, Relationship between plasma measures of oxidative stress and metabolic control in NIDDM, Diabetologia 40 (1997) 647–653.
[15] G. De Mattia, M.C. Bravi, O. Laurenti, M. Cassone-Faldetta, A. Armiento, C. Ferri, F. Balsano, Influence of reduced glutathione infusion on glucose metabolism in patients with non-insulin-dependent diabetes mellitus, Metabolism 47 (1998) 993–997.
[16] M.A. Hofmann, B. Kohl, M.S. Zumbach, V. Borcea, A. Bierhaus, M. Henkels, et al, Hyperhomocystein(e)mia and endothelial dysfunction in IDDM, Diabetes Care 20 (1997) 1880–1886.
[17] M.N. Munshi, A. Stone, L. Fink, V. Fonseca, Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease, Metabolism 45 (1996) 133–135.
[18] E.K. Hoogeveen, P.J. Kostense, P.J. Beks, A.J. Mackaay, C. Jacobs, L.M. Bouter, et al, Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 133–138.
[19] E.J. Giltay, E.K. Hoogeveen, J.M. Elbers, L.J. Gooren, H. Asscheman, C.D. Stehouwer, Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects, Atherosclerosis 139 (1998) 197–198.
[20] V. Fonseca, S. Mudaliar, B. Schmidt, L.M. Fink, P.A. Kern, R.R. Henri, Plasma homocysteine concentrations are regulated by acute hyperinsulinemia in nondiabetic but not type 2 diabetic subjects, Metabolism 47 (1998) 686–689.
[21] E. Ferrannini, S.M. Haffner, B.D. Mitchell, M.P. Stern, Hyperinsulinemia: the key feature of a cardiovscular and metabolic syndrome, Diabetologia 34 (1991) 416–422.
[22] A. Fontbonne, M.A. Charles, N. Thibult, J.L. Richard, J.R. Claude, J.M. Warnet, et al, Hyperinsulinemia as a predictor of coronary heart disease mortality in a healthy population: the Paris prospective study, 15-year follow-up, Diabetologia 34 (1991) 356–361.
[23] V. Sánchez-Margalet, M. Valle, J.A. Lobón, A. Maldonado, F. Escobar, R. Pérez-Cano, R. Goberna, Diminished insulin receptors on erythrocyte ghosts in nonobese patients with essential hypertension independent of hyperinsulinemia, J. Cardiovasc. Pharmacol. 24 (1994) 74–77.
[24] V. Sánchez-Margalet, E. Ramos, J. Mateo, J. Oliván, R. Pérez-Cano, R. Goberna, Normal pancreastatin-like and increased post-glucose insulin levels in young offspring of insulin-resistant non-obese essential hypertensive subjects, J. Endocrinol. 153 (1997) 313–318.
[25] M. Serrano Rios, Relationship between obesity and the incresed risk of major complications in non-insulin-dependent diabetes mellitus, Eur. J. Clin. Invest. 28 (Suppl 2) (1998) 14–17.
[26] G.M. Reaven, Role of insulin resistance in human disease (syndrome X): an expanded definition, Annu. Rev. Med. 44 (1993) 121–131.
[27] G. Paolisso, A. Gambardella, M.R. Tagliamonte, F. Saccomanno, T. Salvatore, P. Gualdiero, et al, Does free fatty acid infusion impair insulin action also through an increase in oxidative stress, J. Clin. Endocrinol. Metab. 81 (1996) 4244–4248.
[28] F.B. Araujo, D.S. Barbosa, C.Y. Hsin, R.C. Maranhao, D.S. Abdalla, Evaluation of oxidative stress in patients with hyperlipidemia, Atherosclerosis 117 (1995) 61–71.
[29] S. Najib, V. Sánchez-Margalet, Homocysteine thiolactone inhibits insulin signaling and glutathione has a protective effect, J. Mol. Endocrinol. 27 (2001) 85–91.
[30] F. Abbasi, F. Facchini, M.H. Humphreys, G.M. Reaven, Plasma homocysteine concentrations in healthy volunteers are not related to differences in insulin-mediated glucose disposal, Atherosclerosis 146 (1999) 175–178.