CLINICAL AND BIOLOGICAL MARKERS OF INSULIN RESISTANCE

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Abstract

Insulin resistance (IR) is lately a much debated and disputed subject. It is already well known that insulin resistance is involved in the metabolic syndrome, dyslipidemia, hypertension, dysfibrinolisis, but also in type 2 diabetes and cardiovascular disease. A number of clinical and biological markers can help us quantify insulin resistance. Among the clinical markers that are most frequently used in practice we can enumerate: waist circumference, waist circumference to hip circumference ratio and waist circumference to height ratio. A newly used marker is neck circumference that is an easier and cheaper method which does not change during the day as other indicators such as waist circumference do. Also Levine et al. observed an association between "chubby cheeks" and increased visceral fat and indicated that these patients have a higher risk of metabolic complications and obesity. Biological markers of insulin resistance like Homeostasis Model Assessment-Insulin Resistance, Homeostasis Model Assessment-Adiponectin, triglycerides, adipocitokines are extremely useful in quantifying IR.

keywords*: Insulin resistance, metabolic syndrome, insulinemia, glycemia*

Background

Insulin resistance is increasingly involved in the metabolic syndrome, dyslipidemia, hypertension, dysfibrinolisis, but also in type 2 diabetes and cardiovascular disease [1]. Shortly after the discovery of insulin and the beginning of using it in the treatment of diabetes it was observed that there is an interindividual variation in the effectiveness of insulin in reducing hyperglycemia.

In 1939 Himsworth and Kerr classified, using the insulin-glucose test, patients in "insulin-sensitive "and" insulin-insensitive". They believed that most cases of diabetes were represented by "insulin-insensitive" types, which now correspond to type 2 diabetes. Yalow and Berson in 1960 discovered radio-immunological determination of insulin and then Karam et al. using this method revealed the existence of insulin plasma values above normal after glucose load in obese individuals without diabetes [2].

Clinical markers

1. Waist Circumference (WC)

Obesity is associated with IR in particular when the fat distribution is central. U.S. National Institutes of Health found that WC and abdominal fat are independent predictor factors for morbidity. A WC greater than 88 cm is considered to be a high risk factor in females and 102 cm in males. WC is measured midway between iliac crest and rib rebord with a centimeter placed horizontally. It is considered that it is a more precise index than BMI in determining risk for diabetes and cardiovascular disease because even at a normal BMI, if WC is increased, the risk of developing cardiovascular disease or diabetes is high. Advantages of this method: a simple measure to implement and it does not involve high costs [3].

2. Body mass index (BMI)

The first step in screening obesity was BMI, which was proposed as a parameter for assessment of weight, more than 150 years ago by Quetelet. BMI is an index calculated from the height and weight values. Although BMI does not measure directly adiposity (fatty tissue), trials have shown a correlation between BMI and direct measurement of fat mass, achieved by underwater weighting or other methods. Therefore, BMI can be considered as an alternative indirect measure of fat mass. In addition, BMI is a very simple and cheap method of analysis for assessing individual disease risk. BMI formula = Weight (W) (kg)/Height (H) ² (meters). Normal values are between 18, 5 and 24, 9 kg/ m². Values below 18,5 are considered underweight, between 30 and 24.9 overweight, 30 - 34,9 first grade obesity, 35 - 39.9 second grade obesity and values ≥ 40 third grade obesity . The advantage of this index is the accuracy height and weight can be measured. Even at a normal BMI a high WC may be considered a risk factor [4].

3. Waist Circumference to Hip Circumference (HC) ratio

Another index used is the WC/HC ratio whose normal values are <0.8 in female patients and < 1 in males patients. Waist circumference is measured midway between iliac crest and rib rebord and hip circumference at the trochanter`s level [5]. WC/HC ratio is strongly associated with BMI and parameters reflecting IR, a high ratio is associated with cardiovascular disease.

4. Neck circumference and bust

Freedman and Rimm believe that increased neck circumference and bust tend to be risk factors for diabetes in women independent of degree of overweight. Levine et al. observed an association between "chubby cheeks“ and increased visceral fat and indicated that these patients have a higher risk of metabolic complications and obesity. Following a study on a sample of 593 subjects from the town of Oulu in northern Finland, it was concluded that neck circumference is related to other anthropometric indices of obesity and also is associated with metabolic syndrome and insulin resistance. Neck circumference was measured with a flexible inch, above the cricoids cartilage with an accuracy of 1 mm. This measurement can be used in screening individuals with IR, is an easier and cheaper method that does not

change during the day as other indicators such as waist circumference do [6].

5. Waist Circumference to Height (H) ratio

It is a simple anthropometric method, by which we can identify people with metabolic risk.

A study in Japan on a sample of 6141 males and 2137 females, showed that the WC/H ratio is an improved method of determining body fat distribution and a better index for prospective studies. This index correlates better with cardiovascular morbidity index and metabolic diseases. Normal values are < 0.5 [7].

Biological markers

1. Insulinemia

Epidemiological studies have correlated serum insulin levels with risk of cardiovascular disease, identifying insulinemia as a marker of IR. Using the oral glucose-tolerance test values of fasting insulin >15 μU/ml with a peak of ≥150 μU insulin/ml and ≥75 μU or/ml at 120 min after glucose load suggest IR.

2. Glycemia/insulinemia ratio.

IR manifests diversely as insulin effects are multiple, all body structures and metabolisms being affected. IR can be highlighted by several methods; the simplest method is calculating the blood glucose (mg/dl) /insulinemia (μU/ml) ratio. Normal values of this ratio are between 6 and 7, in type 2 diabetes the value falls below 6, often ranging between 3 and 5. Another method is to measure the hypoglycemic effect of 0.1U insulin/kg body. Blood glucose is measured initially, then every 3 -5 minutes between 0:15 min. after insulin administration. IR is characterized by low or delayed hypoglycemic response. This test is also called "insulin tolerance test".

3. Homeostasis Model Assessment-Insulin Resistance (HOMA - IR)

The most commonly used in large-scale studies, Homeostasis Model Assessment Method allows the estimation of beta cell function and insulin resistance based on fasting glucose determination and insulinemia. HOMA is ≥ 2.4 in IR.

HOMA - IR% = I x G/22,5

I = basal insulin expressed in μU/ml

G = fasting glucose expressed in mmol/l [5].

4. Homeostasis Model Assessment-Adiponectin (HOMA-AD)

This index is calculated from the product of serum insulin and plasma glucose levels divided by serum adiponectin levels. This study was performed on 117 Japanese subjects with various degrees of glucose tolerance and determined serum adiponectin levels and insulin sensitivity (*M*-value) by using the euglycemic hyperinsulinemic clamp technique. Values were more significantly correlated with HOMA-AD. In subjects with moderate hyperglycemia HOMA-AD showed a more significant correlation with the *M*-value than HOMA-IR [10].

5. Modified Insulin Suppression Test

Another measure of insulin resistance is the modified insulin suppression test developed by Gerald Raven at Stanford University. The test correlates well with the

euglycemic clamp with less operator-dependent errors. Patients initially receive 25 mcg of octreotide (Sandostatin) in 5 ml of normal saline over 3 to 5 min IV as an initial bolus, and then will be infused continuously with an intravenous infusion of somatostatin (0.27 μgm/m ^{2} /min) to suppress endogenous insulin and glucose secretion. Insulin and 20% glucose is then infused at rates of 32 and 267 mg/m ^{2} /min, respectively. Blood glucose is checked at zero, 30, 60, 90, and 120 minutes, and then every 10 minutes for the last half-hour of the test. These last 4 values are averaged to determine the steady-state plasma glucose level. Subjects with an SSPG greater than 150 mg/dl are considered to be insulin-resistant [11].

6. IRI (Insulin Resistance Index)

Insulin secretion during OGTT (oral glucose tolerance test) can be calculated by the following formula: AIR-OGTT = insulinemia / glucose. It can be calculated at 30, 60, 90, 120 minutes during OGTT. Insulin secretor response during TTGIV (intravenous glucose tolerance test) can be calculated based on ratios of areas under the curve (AUC) above baseline, of insulinemia and glucose values AIR-TTGIV = AUC insulinemia / AUC glucose. It can be calculated at 10 min after glucose infusion (insulin secretor early response) or between 10-45 minutes (late insulin secretor response) [2].

7. Euglycemic hyperinsulinaemic (EH) clamp

Euglycemic hyperinsulinaemic clamp (EH) clamp could be translated as "method of euglicemic hyperinsulinemia', but this term is not yet naturalized in our "technical language". This assessment method is based on the amount of glucose needed to maintain normal glycemia during insulin infusion establishing and maintaining a constant, elevated insulinemia. Because infusion technique requires two venous lines and repeated determination of glucose and insulinemia, this method can be used only in research. This method investigates the sensitivity of tissues to insulin action and it is considered the standard method for measuring the IR [5].

8. Quantitative Insulin Sensitivity Check Index (QUICKI)

Although euglycemic hyperinsulinaemic (EH) clamp is the main method to measure insulin sensitivity, it is an expensive method used more in research. "Quantitative Insulin Sensitivity Check Index (Quicki)= **1/[log fasting insulinemia (μU/ml) + log fasting glicemia (mg/dl)]**. QUICKI is ≤ 0,167 in IR [8].

9. Adiponectin

Adipokin biomarkers are secreted by adipose tissue and act autocrine, paracrine or endocrine, adjusting metabolic flux to the amount of energy. Among all the molecules produced by adipocytes, adiponectin has attracted a particular attention, largely because its role of insulin sensitizing, antiatherogenic role and also because adiponectin deficiency is a cause of IR. Serum adiponectin level is 15% higher in women than in men [9]. Low levels of adiponectin were associated, both in animal models and human, with various atherosclerotic risk factors, hypertension, type 2 diabetes, increased insulin resistance, high triglycerides and low HDL, obesity and

inflammation. The favorable effects of adiponectin associated with antiinflammatory effects, antiapoptotic effects, interstitial fibrosis inhibition make from adiponectin a promising therapeutic molecule. Depending on the values obtained we can assess the risk for insulin resistance and artheriosclerosis: values> 10mg/l: low risk of IR, 7-10 mg/l: normal, 4-7 mg/l: increased risk of IR, <4mg/l: high risk of IR [12].

10. Leptin

Leptin was discovered in 1994 by Jeffrey M. Friedman and colleagues at Rockefeller University, through a study on obese mice [13]. Leptin plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin, ob gene product, is a hormone secreted by adipocytes. Animals displaying ob gene mutations are obese and lose weight when leptin is administered. Using a radioimunologic test, serum leptin was measured in 136 subjects with normal weight and 139 obese subjects. In obese subjects leptin levels were 31.3 ± 24.1 ng/ml compared to those with normal weight, where the values were 7.5 ± 9.3 ng/ml and the lowest value seen in an obese subject was 1.7 ng/ml [14]. Plasma level of leptin may be the most useful marker of IR, independent of the insulin plasma level. Leptin normal values <24 ng/ml in women and <10 ng/ml in men [15].

11. Leptin/adiponectin ratio

Levels of leptin increases with obesity and the levels adiponectin decreases in obesity. Leptin/adiponectin ratio in clamp tests is related to Glucose Infusion Rate (GIR), thus this ratio is considered to have a higher accuracy than leptin, adiponectin or HOMA IR taken separately [12].

12. Triglycerides

Dyslipidemia of IR is characterized by increased trygliceridemia, LDL cholesterol and decreased plasma levels of HDL cholesterol. Postprandial hyperlipidemia is common in subjects with IR, likely by reducing chilomicrons lipolysis. TG accumulation was described as dangerous to certain tissues due to the so-called lipotoxicity specifically with action on pancreatic β cells. A direct relationship between IR and postprandial hyperlipidemia was demonstrated by Annuzzi et al. after the hyperinsulinemic clamp studies in type 2 diabetes. Normal levels of triglycerides are less than 150 mg/dl [16].

13. The triglycerides/high-density lipoprotein (HDL) cholesterol ratio.

Triglycerides and the triglycerides/high-density lipoprotein (HDL) cholesterol, are good surrogate markers to identify the IR in obese patients, according to a study Published in Annals of Internal Medicine. In this study BMI was calculated, fasting glucose, insulinemia, lipid and lipoprotein concentrations were dosed in 258 subjects without diabetes, overweight, with BMI ≥ 25kg / m². Most useful markers of IR were serum concentration of triglycerides, triglyceride/HDL cholesterol and insulinemia. The benchmarks were:

- Triglycerides: 1.47 mmol / l (130 mg / dl) (1mmol / l = 18mg/dl)
- Triglycerides/HDL ratio: 3
- Insulin: 109 pmol / l

These benchmarks had a sensitivity of 67%, 57% and 68% and specificity of 64%, 71% and 85% respectively. A triglyceride/ HDL cholesterol ratio increased, correlated with a high cardiovascular risk and IR, this marker being used in practice as an independent factor predictor for mortality and cardiovascular events. Triglycerides/(HDL) cholesterol ratio ≥ 3 in IR [17].

14. Fetuin-A

Fetuin-A is a protein secreted by the liver that binds to its receptor and inhibits insulin action in vitro. High levels of this protein were associated with IR and the age group 70-79 years was associated with increased incidence of diabetes, independent of other markers of IR [18].

15. Estimated Glucose Disposal Rate (eGDR)

IR calculation and is estimated by Glucose Disposal Rates (eGDR) ($mgkg^{-1}min^{-1}$) after the formula has been validated from clamp studies [19].

$$eGDR = 24.31 - (12.22 \times WHR) - (3.29 \times HT) - (0.57 \times HbA1c)$$

where: WHR waist-hip ratio, HT - hypertension history (yes = 1, no = 0), HbA1C - glycosylated hemoglobin A1c.

In this study subjects where divided in three groups according to the likelyhood of insulin resistance: the lowest probability=1, the largest probability=3.

eGDR values based on IR were: group 1= 9.65 ± 2.99 $mgkg^{-1}min^{-1}$, group 2 = 8.02 ± 1.39 $mgkg^{-1}min^{-1}$, and group 3 = 5.68 ± 2.16 $mgkg^{-1}min^{-1}$ [19]. The results were:

- Patients with metabolic syndrome (MS) have lower values than those without MS: eGDR (6.19 ± 1.5 $mgkg^{-1}min^{-1}$ versus 1.6 ± 9.93 $mgkg^{-1}min^{-1}$).
- The eGDR <8.77 $mgkg^{-1}min^{-1}$ has 100% sensitivity and 85.2% specificity for the diagnosis of MS in type 1 diabetes.
- Patients with complications of diabetes have eGDR values <8.16 $mgkg^{-1}min^{-1}$.
- EGDR level is significantly lower in patients with diabetic retinopathy (5.97 ± 1.2 $mgkg^{-1}min^{-1}$), diabetic neuropathy (5.06 ± 0.4 $mgkg^{-1}min^{-1}$), or diabetic nephropathy (5.79 ± 1.5 $mgkg^{-1}min^{-1}$) compared with those without these complications (9.38 ± 2.0 $mgkg^{-1}min^{-1}$, $p < 0.001$; 9.26 ± 2.0 $mgkg^{-1}min^{-1}$, $p < 0.001$ and 9.19 ± 2.2 $mgkg^{-1}min^{-1}$, $p < 0.001$) [20].

16. Alaninaminotransferase (ALT) and the γ-glutamyltransferase (GGT)

Alaninaminotransferase (ALT) and γ-glutamyltransferase (GGT) are significantly associated with IR and metabolic disorders among young obese men in Japan. ALT and GGT can be used as markers of insulin-resistance-associated metabolic disorders in this cohort [21].

17. C-reactive protein

Recently, Winner has shown that high levels of reactive protein C are associated with components of metabolic syndrome such as low HDL cholesterol and increased value of the triglycerides / HDL-cholesterol ratio. C-reactive protein (CRP) is positively correlated with obesity, with an increased BMI and with the metabolic syndrome. Values > 3mg/l are correlated with IR.

18. Rezistin

Rezistin was associated with IR in obese mice. Serum levels of rezistin were correlated with IR, low HDL cholesterol, and high sensitivity CRP in the general population of Japan. Rezistin was correlated with the metabolic syndrome and with the presence of microangiopathy in type 2 diabetes [22].

19. Steroid hormones have been associated with markers of IR [23]

Glucocorticoids' effects antagonize those of insulin. Also, visceral adipose tissue has more glucocorticoids' receptors than the subcutaneous fat. In obese patients, the level of growth hormone (STH) is low, leading to an increased 11 beta-hydroxysteroid dehydrogenase-1 activity (11 HSD1) and more cortisol converted from cortisone. Omental adipose tissue contains high levels of 11 HSD1, favoring the conversion. The insulin resistant subjects have high levels of urinary cortisol secondary to the lower levels of corticosteroid-binding globulin. Routine dosages of cortisol are recommended only when we suspect the Cushing's syndrome. Level of sex hormone binding proteins are usually reduced in IR, this condition leading to increased levels of free testosterone, hyperandrogenism, hirsutism expressed by acne and irregular menses in women. In men, an increased aromatization of androstendion in the adipose tissue leads to the increase of serum estradiol, causing gynecomastia.

20. Insulin-like growth factor (IGF1)-binding protein

Insulin-like growth factor (IGF-1)-binding protein may be affected by obesity and IR. These children and adolescents usually present low values of IGF-binding protein, although the total value to IGF-1 is normal. This imbalance may lead to increased tissue bioavailability for IGF-1, increasing the hypoglycemic effect of insulin. These patients may present an accelerate linear growth and pseudo-acromegaly when the insulin resistance is severe.

21. Ghrelin

Ghrelin is a hormone produced mainly by P/D1 cells lining the fundus of the human stomachs which increases appetite and stimulates gastric emptying. Galli-Tsinopoulou et al. have demonstrated that ghrelin, in obese children with IR, is significantly suppressed immediately after the intake of the glucose in the oral glucose tolerance test; ghrelin may be used as a marker of IR used in research.

22. Retinol-binding protein 4

Retinol-binding protein 4 is a newly identified adipokin which is secreted by the liver and adipocytes. A recent study in China showed a positive association with the metabolic syndrome, serum concentration of retinol-binding protein and its ratio to serum retinol correlated with obesity and IR.

23. Interleukins

IL-6 was associated with IR and may interfere with insulin signaling by inhibiting adipogenesis and adiponectin secretion. An increased level of IL-6 is present in obesity. IL-10 carries a protective role against atherosclerosis development in experimental animals; IL-10 was negatively linked with obesity, these subjects showing low values. TNFα is another cytokine that is related to IR.

This cytokine induces lipolysis in adipose tissue, inhibits insulin signaling and influences the expression of genes that are important for the function of adipose cells. TNFα may also inhibit adiponectin synthesis and the release of fatty acids from fatty tissue, which affects the whole body energy homeostasis and insulin sensitivity.

Intracellular adhesion molecule-1 and vascular adhesion molecule E-selectin are biomarkers of the endothelial dysfunction. Obese children with IR may have increased levels of these markers [24].

REFERENCES

1. **Miriam Cnop, Melinda J. Landchild, Josep Vidal, Negar G. Knowles, Feng Wang, Rebecca L. Hull, Edward J. Boyko, Barbara M. Retzlaff, Robert H. Knopp, Steven E. Kahn, Darcy R. Carr, Peter J. Havel, Carolyn E. Walden**. The Concurrent Accumulation of Intra-Abdominal and Subcutaneous Fat Explains the Association Between Insulin Resistance and Plasma Leptin Concentrations. *Diabetes*. 2002; 51(4).

2. **Radu Lichialdopol**. *Insulin secretion and insulin resistance in etiopathogenesis of type 2 diabetes*. 1999:76-78.

3. **Kristina Jackson Behan, PhD, MT(ASCP), Justice Mbizo, DrPH**. The Relationship Between Waist Circumference and Biomarkers for Diabetes and CVD in Healthy Non-Obese Women. *The Pensacola Study. Laboratory Medicine*. 2007; 38(7): 422-427.

4. **George A.Bray, Donna H.Ryan**. *Overweight and the Metabolic Syndrome: from bench to bedside.* 2006; ISBN 0-387-32163-2: 176-178.

5. **C.Ionescu Târgovişte, R.Lichialdopol, Cristian Guja**. *Diabetes.* 2007; ISBN 978-973-7928-16-0:48-49.

6. **M Laakso, V Matilainen and S Keinänen-Kiukaanniemi**. Association of neck circumference with insulin resistance-related factors. *International Journal of Obesity*. 2002; 26(6): 873-875.

7. **S D Hsieh, H Yoshinaga, T Muto.** Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *International Journal of Obesity (2003) 27, 610–616.*

8. **B. Antuna-Puente, M. Faraj, A.D. Karelis, D. Garrel, D. Prud'homme, R. Rabasa-Lhoret, J.-P. Bastard. HOMA or QUICKI**: Is it useful to test the reproducibility of formulas? *Diabetes Metabolis*. Volume 34, 3, 294-296 (juin 2008).

9. **Claudia Menzaghi, Vincenzo Trischitta, and Alessandro Doria**. Genetic Sway of Adiponectin on Insulin Resistance, Type 2 Diabetes, and CVD: Adiponectin: A Salutary Adipokine. *Diabetes*. 2007; 56(5):1198-1209.

10. **Yamasaki A, Masanori Emoto B, Michio Shimabukuro C, Shinichiro Ueda D, Tohru Funahashi E, Yuji Matsuzawa F.** Corrigendum to ''A novel index of insulin resistance determined from the homeostasis model assessment index and adiponectin levels in Japanese subjects'' *Diabetes Res. Clin. Pract*. 77 (2007) 151–154

11. **T Wallace, J Levy, Matthews D** Use and abuse of HOMA modeling". *Diabetes Care* **27** (6): 1487–95. doi : 10.2337/diacare.27.6.1487 . PMID 15161807 . (2004)

12. **Naohisa Oda, Shigeo Imamura, Takashi Fujita, Yuka Uchida, Kazumichi Inagaki, Hiroaki Kakizawa, Nobuki Hayakawa, Atsushi Suzuki, Jun Takeda, Yukio Horikawa, Mitsuyasu Itoh.** The ratio of leptin to adiponectin can be used as an index of insulin resistance. *Metabolism,* Volume 57, Issue 2, February 2008, Pages 268-27.

13. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** (December 1994). "Positional cloning of the mouse obese gene and its human homologue". *Nature* 372 (6505): 425–32.

14. **RV Considine, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ & Bauer TL .** "Serum Immunoreactive-Leptin Concentrations in

Normal-Weight and Obese Humans". *N Engl J Med* 334 (5): 292–295.

15. Nain-Feng Chu, Jin-Biou Chang and Shih-Ming Shieh. *Obesity Research*, april 2003,11 (4): 532-540.

16. Roberto Miccoli; Cristina Bianchi; Giuseppe Penno; Stefano Del Prato. Insulin Resistance and Lipid Disorders. 2008, ***Future Lipidology*. 3(6):651-664.**

17. Laurie Barclay, MD. Triglycerides and TG-HDL Ratio Help Identify Insulin Resistance in Overweight Patients. *Intern Med*. 2003;139:802-809.

18. Laurie Barclay. Fetuin-A Linked With Incident Diabetes. JAMA. 2008;300:182-188.

19. Katherine V. Williams, John R. Erbey, Dorothy Becker, Silva Arslanian, and Trevor J. Orchard. Can Clinical Factors Estimate Insulin Resistance in Type 1 Diabetes. *Diabetes*. 2000; 49(4):626-32.

20. Eric S. Kilpatrick, Alan S. Rigby, Stephen L. Atkin. Insulin Resistance, the Metabolic Syndrome, and Complication Risk in Type 1 Diabetes "Double diabetes" in the Diabetes Control and Complications Trial. doi: 10.2337/dc06-1982 *Diabetes Care* March 2007 vol. 30 no. 3 707-712.

21. Teruhiko Koike, Maiko Miyamoto and Yoshiharu Oshida. Alanine aminotransferase and γ-glutamyltransferase as markers for elevated insulin resistance-associated metabolic abnormalities in obese Japanese men younger than 30 years of age. 2009. *Obesity Research* &Clinical Practice.

22. Onuma H, **Osawa H**, **Makino H**. Role of resistin in insulin resistance. *Rinsho Byori*. 2008. Aug;56(8):698-704 .

23. Kerstin Brismar and Sven E. Nilsson. Interrelations and associations of serum levels of steroids and pituitary hormones with markers of insulin resistance, inflammatory activity, and renal function in men and women aged >70 years in an 8-year longitudinal study of opposite-sex twins; *Gender Medicine*, Volume 6, Part 1, 2009 , 123-136.

24. Francisca Eyzaguirre, Verónica Mericq, Insulin Resistance Markers in Children, *Hormone Research in Pediatrics* 2009.Vol. 71, No. 2, 65-74.

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