Titolo rivista/libro: Alternative therapies in health and medicine

Titolo articolo/sezione: A Clinical Evidence of a Correlation Between Insulin Resistance and the ALCAT Food Intolerance Test

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ISSN: 1078-6791

DOI:

Anno: 2019

Volume: 25

Fascicolo: 2

Editore:

Pag. iniziale: 22

Pag. finale: 38

ORIGINAL RESEARCH

A Clinical Evidence of a Correlation Between Insulin Resistance and the ALCAT Food Intolerance Test

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ABSTRACT

Insulin resistance (IR) is defined as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization. Several mechanisms have been proposed as possible causes underlying the development of IR and the IR syndrome.

IR occurs as part of a cluster of cardiovascular-metabolic abnormalities commonly referred to as "The Metabolic Syndrome." This may lead to the development of type 2 diabetes, accelerated atherosclerosis, hypertension, dysmenorrhea, hirsutism, and polycystic ovarian syndrome, depending on the genetic background of the individual developing IR.

The aim of this study was to assess, in 123 female and 35 male (mean age, 42 y ± 10.3; range 19-75 y) volunteers) whether IR could be partly related to a dietary sugar intolerance and whether there could be a correlation between the ALCAT intolerance test and a mutation of the *TCFTL2* gene (it promotes the trascription of the proglucagone and plays a key role in the development of the Langherans islands).

Results evidenced that subjects with an intolerance to sugar, also showed a statistically significant complete or incomplete alteration of the *TCFTL2* genetic test.

Based upon these findings, our study demonstrated that there is a clinical correlation between the ALCAT food intolerance test and the IR. The positivity to the ALCAT test of one of the sugars tested (fructose, sugar cane, and sugar beet) indicates, in the majority of the subjects, the presence of a mutation of the gene *TCF7L2* and could contribute to the prevention and treatment of the IR. (*Altern Ther Health Med.* 2018;25(2):22-28.)

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INTRODUCTION

Growing evidence underscores the primary role of factors such as body weight and glycemic control in health and recovery from illness. Carbohydrate intake is an important nutritional issue in the diet, yet it can adversely affect major body organs and tissues if resulting plasma glucose becomes too high.

The principal aim of this paper was to investigate a putative association between IR, through detection of the *TCF7L2* gene, which promotes the transcription of proglucagon and plays a role in the development of the Langherans islands and intolerance to glucose, via the ALCAT intolerance test. *Glucose intolerance* is a general term

referred to higher than normal blood glucose levels, such as hyperglycemia. Conditions that can be considered as glucose intolerance include impaired fasting glucose, impaired glucose tolerance, prediabetes, and type 2 diabetes. Symptoms of glucose intolerance may match those of type 2 diabetes, such as dry mouth, extreme tiredness, feeling thirsty, blurred vision, drowsiness, frequent need to urinate, and loss of muscle mass. IR is clinically defined as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and use in an individual as much as it does in a normal population. Insulin action is the consequence of insulin binding to its plasma membrane receptor and is transmitted through the cell by a series of protein-protein interactions.

Several mechanisms have been proposed as possible causes underlying the development of IR and the IR syndrome. These include (a) genetic abnormalities of 1 or more proteins of the insulin action cascade, (b) fetal malnutrition, and (c) increases in visceral adiposity. IR occurs as part of a cluster of cardiovascular-metabolic abnormalities commonly referred to as *the IR syndrome* or *the metabolic syndrome* (MetS). This cluster of abnormalities may lead to the development of type 2 diabetes, accelerated atherosclerosis, hypertension, dysmenorrhea and hirsutism and/or polycystic ovarian syndrome depending on the genetic background of the individual developing the IR,[1] even though data of a correlation between the *TCF7L2* gene and the micropolycystic ovary are still controversial. IR and other endocrine factors of the MetS induce vascular endothelial lipid profile alterations, which directly or not may play a major role in the atherosclerosis progression.[2-4]

Recently, it has been suggested that MetS may cause brain damage, as chronic hyperglycemia and IR represent neuronal death risk factors due to a putative increase in the oxidative stress and/or inflammatory response, therefore negatively influencing cognitive processes.[5-6]

Several data show that a restriction of carbohydrates may represent a valuable and effective way of counterbalancing and reversing the MetS, thus reducing the cardiovascular risk.[7-8]

Human studies suggest a correlation between variants of the gene *TCF7L2* and type 2 diabetes. The *TCF7L2* gene is located on the long (q) arm of chromosome 10 at position 25.3.[9-11]

Consequently, IR tendency may be outlined through genetic tests, such as gene *TCFTL2* mutation, particularly through *TCFTL2* mutation analysis. In fact, its mutations are responsible for an insulin-reduced secretion.

Genetic tests have been performed via DNA analysis, whereas intolerance food test has been processed through ALCAT test. The ALCAT test may be considered ideal to identify and eliminate individual food stimuli that may act as triggers for the cellular nonspecific immune response. Although the test is not diagnostic, studies have established that it can be a useful screening tool for the identification of foreign substances that may trigger immune cell activation, particularly of neutrophils, leading to inflammatory disorders.[12]

Based on these findings, our aim was to assess whether IR could be partly related to a dietary sugar intolerance in men and women and whether there could be a correlation between the ALCAT test and a mutation of the *TCFTL2* gene.

MATERIALS AND METHODS

Subjects

A total of 158 adult volunteers, 123 female and 35 male (mean age 42 y ± 10.3; range, 19-75 y) were solicited from the Preventive and Genetic Medicine Institute (IMGEP, Milan, Italy) and the Department of Experimental Medicine and Public Health (University of Camerino, Camerino, Italy). Participants gave written informed consent and they have been chosen from a random Italian population of subjects, casually selected from different regions of the country, showing no major acute or chronic pathologies, under no pharmacological prescriptions. On the other hand, it is worth noting that the majority was, every so often, suffering from a generalized gastrointestinal distress with all of them being overweight. They provided samples via each collection method to control for intraindividual variation in collection quality and amount. Two buccal and mouthwash samples were collected via the swab, cytobrush, and oral rinse, so that one DNA sample could be isolated using a protocol typically used for that method.

Swab Brush

Each participants were asked to (1) not brush his/her mouth/teeth for at least 2 hours prior to testing, and (2) rub 1 swab brush (Master Amp Buccal Swab Brush, nylon bristle buccal cell collection brush, Epicentre Technologies, Madison, WI, USA) against his/her right cheek for 60 seconds. Donors were reminded to use Latex examination gloves and to turn the swab brushes to use both sides of the swab. To maximize buccal cell yield, swab brushes were immediately air dried and capped back. They were then shipped to the IMGEP laboratory as specimens.

Testing Protocol

Genetic Test. IR genetic test is a predictive test based upon DNA analysis. Real time method with TaqMan probes was used for the analysis of polymorphisms rs7903146 and rs12255372 in *TCF7L2* gene.

More specifically, the reaction mix for the analysis of rs12255372 for each sample consists of 5 mL of TaqMan Genotyping Master Mix (Applied Biosystems cod 4371355), plus 0.25 mL of primers and probes TaqMan 40x (C__291484_20), plus 3,75 µL of water, plus 1 µL of DNA (10ng/µL). For the G (WT) and T allele respectively VIC and FAM reporter were used.

In the same plate, DNA with G genotype genotype (VIC reporter), GT genotype (VIC and FAM reporters) and T genotype (FAM reporter) were introduced together with a blank, as internal controls.

For the analysis of polymorphisms rs7903146, the amount and quality of reagents were the same as those

aforementioned with the exception of the probes that in this case were TaqMan 40× (C__29347861_10) and of the reporters: for the C allele (WT) VIC reporter and for T allele FAM reporter.

As aforementioned, DNA with C genotype (VIC reporter), CT genotype (VIC and FAM reporters), and T genotype (FAM reporter) were introduced in the same plate as internal controls.

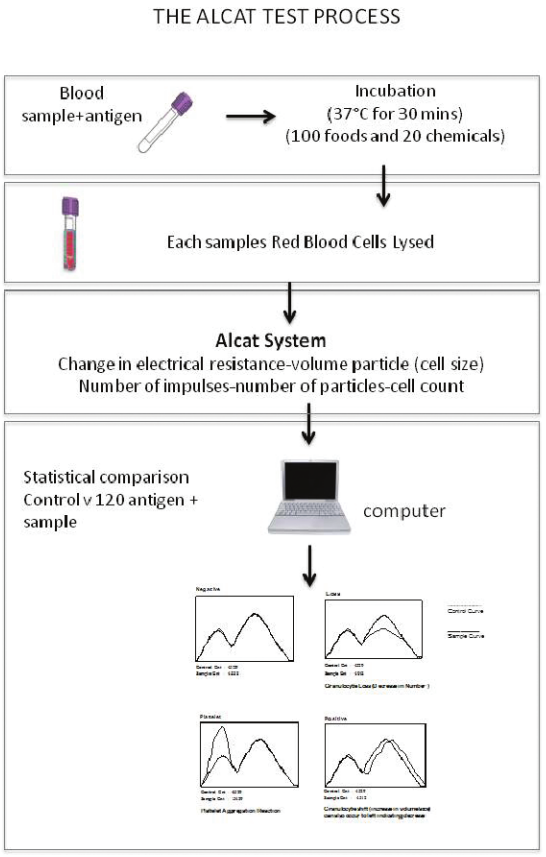
The samples were heated at 60°C for 30 seconds, then 10 minutes at 95°C and then exposed to a 45 amplification cycles for 15 seconds at 95°C, followed by a 1 minute at 60°C, with a last cycle at 60°C for 30 seconds. Figure 1 outlines the 3 putative genetic results.

The genotypes CT/GT and TT/TT are prone to develop an increase in IR, type 2 diabetes, and metabolic syndrome.

Figure 1. Putative Genetic Results

Homozygote **TT/TT**	Alteration–complete mutation
Heterozygote **CT/GT**	Alteration–incomplete mutation
Homozygote **CC/GG**	Normal genetic assessment

Figure 2. The ALCAT Test Process



Abbreviations: ALCAT, antigen leukocyte antibody test; CNT, count.

ALCAT Test

To evaluate the sensitivity to sugars (fructose, sugar cane, and sugar beet), the ALCAT test has been used. It has to be stressed out that the ALCAT test is not a diagnostic tool for food allergies, as these are immunoglobulin E-mediated. It is currently considered a medical device that offers information upon putative food intolerances and/or chemicals.

The ALCAT test uses a specially designed particle counter (hematology analyzer with an automated assay sampler) and food test agents to semiqualitatively measure white blood cell reactivity, if any, to each agent analyzed (Figure 2). The degree of reactivity is determined by comparing a baseline distribution curve (of the white cells) against the distribution curve generated by the analysis of each test agent/blood sample, and calculating the absolute differences between the curves and the standard deviation (SD).

Each sample is processed in duplicate. Any reactivity under SD1 will be considered as nonreactive (NEG); reactivity between SD1 and SD2 will be considered as marginally reactive (RANGE 1+); reactivity between SD2 and SD3 will be considered reactive (RANGE 2+); and finally, reactivity above or equal to SD3 will be considered markedly reactive (RANGE MPOS).

There is evidence demonstrating the ALCAT test to be effective in improving body mass index and/or scale weight. Studies have reported that participants who followed the ALCAT plan achieved rather dramatic changes in their body composition, showing that 98% of the subjects following the ALCAT plan either lost scale weight or improved their body composition.[13]

Other studies reported that iso-caloric food elimination diets, based on ALCAT test outcomes, promoted enhanced weight loss, comprised more of adipose tissue, rather than muscle mass, as determined by DEXA studies in a population of refractory weight loss subjects.[14]

A reproducibility of 92.07% of ALCAT test was reported by Neetling et al, at the University of the Free Orange State. In addition, further data were acquired in South Africa, which makes it an acceptable screening model for intolerance testing in humans. In Addition, a Norwegian study reported the ALCAT test to be >90% reproducible.[15,16]

Fell et al[17,18] reported an 83.4% correlation between ALCAT test results and double blind oral challenges as determined by careful clinical evaluation in statistically significant number of patients exhibiting food sensitivity related symptoms, such as migraines, and irritable bowel syndrome, eczema and other conditions, which are often observed as comorbidities in obese patients.

The histograms present different patterns varying from patients to patients. The distribution of a normal blood sample shows 2 picks, of which the one on the right may have the same height or slightly higher/lower than the one on the

left (with the exception of subjects under younger than 3 years).

As reported in the aforementioned panel, the histogram shows, in the up far left area, the platelet agglutination (if any), a pick corresponding to lymphocytes on the left, a mixed cell region between the 2 picks and, to the up far right, the granulocytes' pick.

The putative platelet aggregation may also appear as an increase of cells in the lymphocyte fraction.

When it is not accompanied by a simultaneous reduction in the number of granulocytes (the right pick), this reaction may be interpreted as a platelet aggregation.

Five different reactions may verify the following:

1. Increase of the cellular volume, the right or the left pick shift toward the right side.
2. Partial degranulation, the right or left pick shift toward the left side.
3. Cellular lysis, the right or left pick are lower due to a cell loss.
4. Platelet aggregation, a pick in the far up left appears in the histogram.
5. No reaction, the picks of the sample and control overlap each other.

The results of the ALCAT test are displayed using the "code color."

Test Results

Four distinct areas exist:

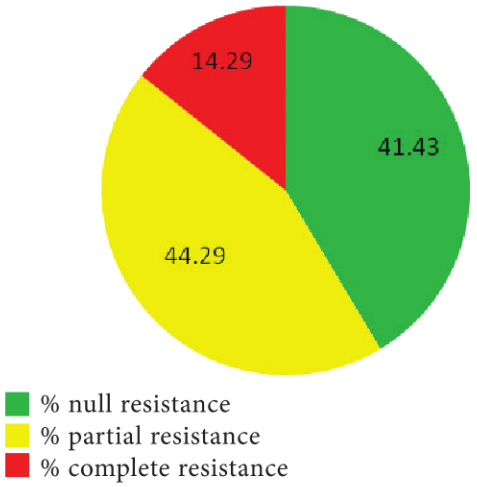
1. Red: Foods listed indicate a severe reaction and should be strictly avoided at all costs for a minimum of 6 months.
2. Orange: Foods listed indicate a moderate reaction should be strictly avoided for a minimum of 3 to 6 months.
3. Yellow: Foods listed indicate a mild reaction, with an asterisk next to the food, should be avoided if possible, for 3 months, especially if there are few red and orange foods.
4. Green: Foods listed indicate no reaction and can be eaten freely on a rotation basis, no more than every other day.

The 2 analyses (the genetic and the ALCAT test) have been compared to verify whether a difference between sugar intolerance and *TCF7L2* gene mutation may exist.

RESULTS

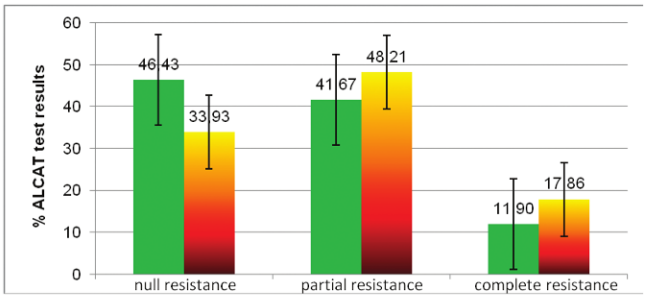
Results of the genetic test are reported in Figure 3. Findings outline that, among the examined subjects, 41.43% show a normal genetic assessment (green color). The highest percentage is represented by the heterozygote genotype associated with an incomplete mutation alteration (44.29%), whereas the complete mutation has been found in a limited sample (14.29%).

Figure 3. Genetic Test Results



Abbreviation: ALCAT, antigen leukocyte antibody test.

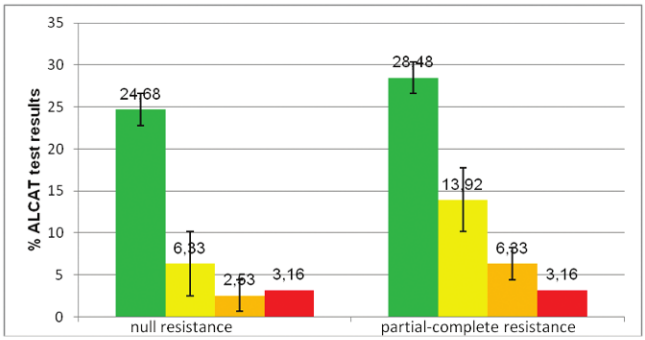
Figure 4. Results of the Genetic Test Compared With the ALCAT Test



Abbreviation: ALCAT, antigen leukocyte antibody test.

Note: In case of a partial-complete insulin-resistance, response percentage to the ALCAT test showing a sugar intolerance is 23.41% when compared with 12.02% observed with no insulin-resistance (Figure 5).

Figure 5. Comparison Between Partial to Complete or Null Insulin Resistance With ALCAT Test Results



Abbreviation: ALCAT, antigen leukocyte antibody test.

Comparing the results of the 2 tests, Figure 4 shows that, when negative (*TCF7L2* normal gene assessment), a higher percentage of subjects with no intolerance to sugars is present. On the contrary, when a complete mutation of the *TCF7L2* is evidenced, a reduction in the percentage of subjects showing no intolerance to sugars is found (green bar of the ALCAT test). Nevertheless, a negative ALCAT (green bar) has whatsoever been evidenced even in presence of a partial and/or complete mutation. This finding could be somehow related to a series of circumstances, such as a previously determined diagnosis or to a good quality standard life style. Therefore, it is reasonable to hypothesize that these patients will not show the pathology, even though putatively genetically predisposed to the insulin-resistance pattern. To extensively analyze the correlation between the IR and the ALCAT test results, a statistical analysis has been applied.

The evaluation of the correlation between the "color" group of test results and the IR was performed by χ^2 test (Figure 6). Results show a higher percentage of "patients" with null resistance (80%) in the green group, even if the results are not statistically significant; this is probably due to the small number of patients recruited in relation to the high variability of the parameters studied.

In addition, attributing a score ranging from 1 to 4 to the color groups (1, green; 4, red) and a value to the resistance (0 to null, 50 to partial and 100 to complete) we also performed a Pearson correlation test. Test showed a tendency to increase, even though the results were not statistically significant (P=.54; Figure 7).

When compared with the genetic test results, the ALCAT analysis further shows differences between total to partial or null insulin-resistance patients. Specifically, in the case of patients with null resistance, the 27.27% of the samples shows no intolerance to total sugars (green bar), with the remainders evidencing a mild (9.09% yellow bar) to a severe intolerance (red bar 27.27%; Figure 8).

Among subjects evidencing a total to partial resistance to the genetic test, the ALCAT results show an increase of the percentage associated to intolerance (form mild to severe). As shown, there is a difference in terms of response amongst the several types of sugars analyzed.

When separately analyzing the sugars tested, it could be noted that fructose and beet appear to be, for the major part, responsible for total to partial intolerance, either in the group of patients with null resistance or in the total-to-partial resistance group. In fact, a diet high in sucrose or in high-fructose sweeteners used in beverages and processed foods starts a chain of metabolic events, which can give rise to hypertriacylglycerolemia, hyperinsulinemia, IR, and elevated blood pressure. Fructose bypasses the step in glucose metabolism, at phosphofructokinase, where control is exerted on the rate of catabolism of glucose. It is rapidly taken up in the liver and converted to intermediate metabolites in the glycolytic pathway depleting cells of inorganic phosphate leading to inhibition of phosphofructokinase. Inhibition of this enzyme blocks the processing of glucose through the glycolytic pathway. This results in the shunting of glucose through the polyol pathway resulting in the formation of more fructose and a build-up of intermediate metabolites such as glyceraldehyde, glyceraldehydes-3-phosphate and dihydroxyacetone phosphate, with further metabolism to methylglyoxal, a highly reactive ketoaldehyde. Methylglyoxal itself inhibits the glycolytic pathway which leads to further IR and production of excess aldehydes.[19,20] On the other hand, sugar cane appears to be the most tolerated in both groups.

Figure 6. χ^2 Test

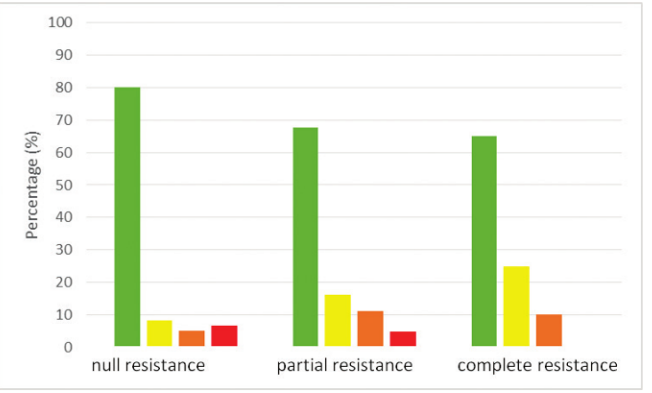
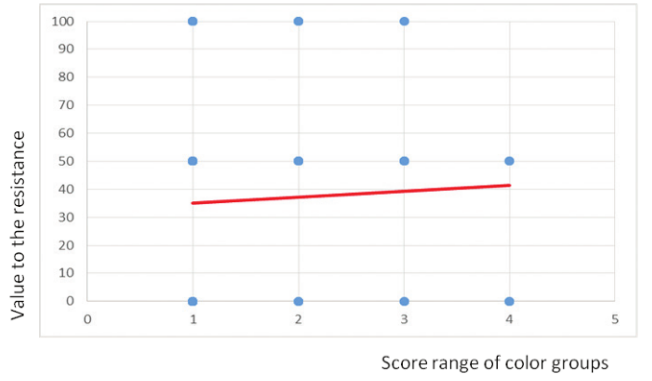


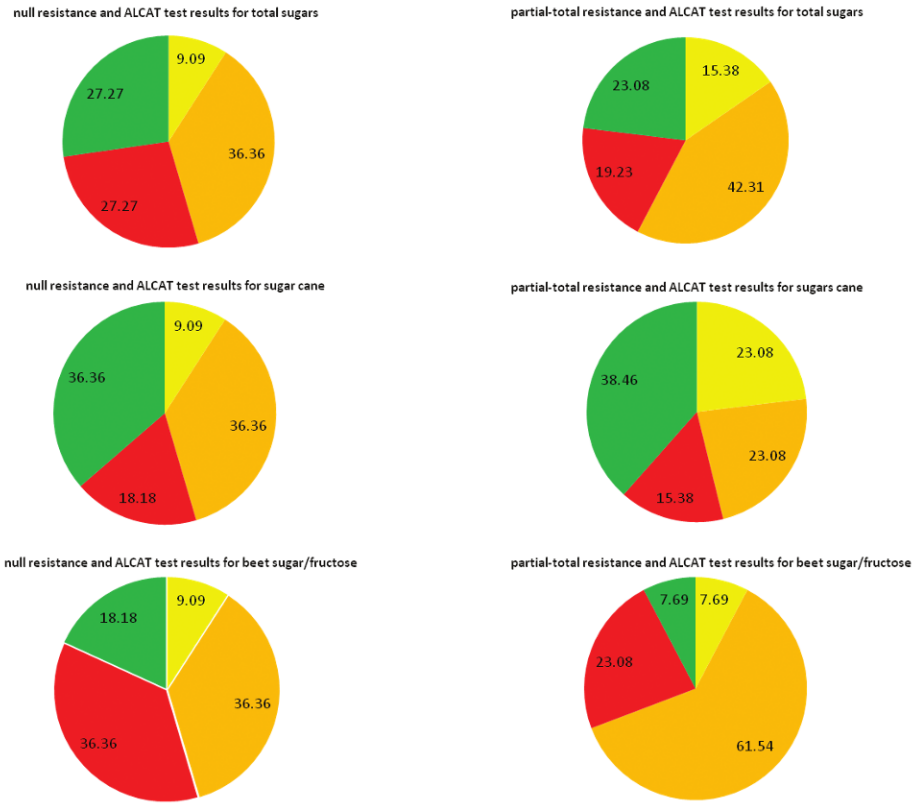
Figure 7. Pearson Correlation Test



DISCUSSION AND CONCLUSIONS

Overweight and more seriously obesity are a major public health problem in most societies and more recently, among the Italian population. The associated health risks and diseases represent a tremendous drain on the economy and strongly affect the quality of life. The physiopathological mechanisms developing MetS are clearly linked to inflammation, confirmed by several studies.[20-22] Food inflammation, better defined as immune-flogosis, is a subclinical state of a more general systemic inflammation, which may generate trigger factors for further pathological conditions. The relationship between food inflammation and IR has become crystal clear when facing patients with clinical symptoms of a pre-MetS, observing his/her phenotype. Metabolic indices alterations, such as weak dyslipidemia, increase of the abdominal circumference, borderline hypertension, and modified glycemic parameters

Figure 8. Comparison Between ALCAT Test Results and Insulin Resistance, as Analyzed by Sugar Types



Abbreviation: ALCAT, antigen leukocyte antibody test.

may be considered as preclinical patterns of a putative MetS. Moreover, oxidative metabolism is one of the factors leading to mitochondrial dysfunction. It has been shown that there are genetic alterations related to MetS and gene expression regulation, thus yielding a reduction of the protein transcription involved in the glucose metabolism.[23] Genetic and cell alterations promote fatty acid oxidation, which may cause mitochondrial modifications, as diagnosed in IR patients. Hormonal response to food is physiologically well known, because it is strongly related to the type of nutrients introduced on a daily basis. Hormones, receptors and ligands, are responsible for a normal insulin secretion, therefore immune-flogosis related to food, is capable of ultimately altering the insulin response.

Based on these findings, in this study, we have highlighted that there is a clinical correlation between the ALCAT food intolerance test and the IR. The positivity to the ALCAT test of one of the sugars tested (fructose, sugar cane and sugar beet) indicates, in the majority of the subjects, the presence of a mutation of the gene *TCF7L2*. A negative reaction to the ALCAT test of the sugar group may not exclude the presence of minor gene mutations putatively due to (1) the subject could have been previously exposed to a limited sugar intake in his/her daily diet, (2) a previous diagnosis, and (3) a regular daily exercise training (i.e. 30 minutes walking) combined with a proper nutrition.

Based on the outcomes of the statistical analysis, further studies need to be performed either to widen the number of patients or to gain more insight regarding the correlations between food intolerances and IR. Further investigations are needed to evaluate which mechanisms may interfere in the relationship between sugar intolerance and gene susceptibility.

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