

METHODS FOR DETECTING LOW GRADE INFLAMMATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of European Patent Application No. 10192405.8, filed November 24, 2010, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods of detecting low grade tissue inflammation.

BACKGROUND

Low grade tissue inflammation is increasingly recognized as one of the main factors associated with insulin resistance and diabetes in obese subjects (1, 2). Tissue inflammation is thought to be mainly mediated by factors secreted by adipose tissue (e.g. TNF α , IL6, MIF, CSF1, MCP1, fatty acids) and consists of a series of cellular responses, such as intracellular pathways activation and endoplasmic reticulum stress responses, that are responsible for the amplification of the inflammatory status and for the alteration of the tissue metabolism. For instance, TNF α mediates the inactivation of the insulin receptor signaling through IKK β and JNK-mediated IRS serine phosphorylation. At the same time IKK β and JNK also activate NF κ B and AP-1 pathways, thus mediating amplification of cytokines release. NF κ B and AP-1 –mediated cytokine release is also the ultimate step of other pathways triggered by other circulating factors, such as free fatty acids through Toll-like-receptor, or elevated glucose through induction of endoplasmic reticulum stress response. Low grade inflammation is also associated with endothelial dysfunction which increases cardiovascular risk in obese and insulin resistant subjects.

The evaluation of low grade inflammation in tissues could be a useful approach for the understanding of insulin sensitizer's mode of action, and also a potential approach to phenotype sub-populations within the pre-diabetic and diabetic population for personalized medicine. However the access to tissue biopsies in a clinical setting poses several problems. Hence, it is necessary to profile a surrogate system for the evaluation of inflammatory pathways regulation for pharmacodynamic and patient stratification purposes.

Peripheral Blood Mononuclear cells (PBMCs) have been explored in the recent past as a potential surrogate for the study of NF κ B-related pathways regulation in insulin resistant states. Expression of genes related to NF κ B pathway in blood cells was shown to (i) reflect whole-body chronic inflammation and to recapitulate tissue inflammation in T2D and/or co-morbidities

(hypertension, diabetic nephropathy) (3-12) and (ii) to be modulated by life-style and therapeutic intervention (e.g. obesity, hypertension) (3-12). In addition, innate immune system activation (e.g. granulocytes) has been shown to be associated with obesity (13, 14).

It is desirable to provide simplified methods for detecting the presence of low grade inflammation in patients to aid in identifying such patients and in providing effective therapeutic intervention.

SUMMARY

The present invention provides methods of detecting the presence of low grade inflammation in a patient by analyzing a whole blood sample taken from the patient. In some embodiments, the patient is an obese patient and/or a patient suffering from Type II diabetes. In some embodiments, the patient is an insulin resistant patient.

One aspect of the invention provides for a method for detecting the presence of low grade inflammation in a patient comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient. A change in expression profile of the set of genes as compared to a healthy control sample indicates the presence of low grade inflammation in the patient.

Another aspect of the invention provides for a method of identifying a patient who may benefit from treatment with an insulin sensitizer comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the set of genes as compared to a non-insulin resistant control sample indicates that the patient may benefit from treatment with an insulin sensitizer.

Another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the set of genes as compared to a non-insulin resistant control sample indicates that the insulin sensitizer treatment is not effective.

In one embodiment of the above aspects, the set of genes comprises one or more genes showing a correlation between whole blood and adipose tissue expression. In one embodiment, the genes are selected from the group consisting of resistin, leptin, FoxP3, CD79A and CTLA4. In one embodiment, the set of gene comprises two, three, four, or all five of resistin, leptin, FoxP3, CD79A

and CTLA4.

In one embodiment of the above aspects, the set of genes comprises one or more genes showing an association with insulin resistance. In one embodiment, the genes are selected from the group consisting of IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, the set of gene comprises two, three, or all four of IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, insulin resistance is determined by hyperinsulinemic euglycemic clamp or by homeostasis model assessment as an index of insulin resistance (HOMA-IR).

In one embodiment of the above aspects, the set of genes comprises one or more genes associated with elevated BMI. In one embodiment, the genes are selected from the group consisting of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS. In one embodiment, the set of gene comprises two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or all thirteen of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS.

In one embodiment of the above aspects, the set of genes comprises one or more genes from Table 1, wherein the genes are related to inflammation and NFkB pathway.

In one embodiment of the above aspects the patient is suffering from a metabolic condition, such as type 2 diabetes, obesity, insulin resistant states, nonalcoholic steatohepatitis (NASH), or nonalcoholic fatty liver disease (NAFLD).

In one embodiment, the expression profile in the above aspects is determined by measuring mRNA expression level. In one embodiment, the mRNA expression level is measured using qRT-PCR.

In one embodiment, the change in expression level of the set of genes in the test sample is at least about a 1.5 fold difference as compared to the control sample.

In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table 1.

Another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising the steps of a) determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, b) comparing the expression profile of the set of genes to the

expression profile of the set of genes from reference sample of whole blood taken from the patient prior to treatment with the insulin sensitizer, and c) determining that the therapy is effective when the expression profile of the genes in the test sample is more similar than the expression profile of the genes in the reference sample to the expression profile of non-insulin resistant control sample. In one embodiment, the set of gene comprises one or more genes selected from the groups above. In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Graphical representation of the differential gene expression patterns between obese and lean patients.

Figure 2. Plot showing whole blood Spearman correlation of gene expression patterns from obese and lean patients.

Figure 3. Graphs showing differential regulation of certain genes (Δ CT) in obese and lean subjects.

Figure 4. Graphs showing the gene expression levels of blood-cell specific genes in obese and lean subject.

Figure 5. Graph comparing gene expression determined in PBMC and whole blood samples.

Figure 6: Table with panel of genes showing correlation between adipose and whole blood

Figure 7: Graphs of genes associated with insulin resistance in type 2 diabetes (T2D), as determined by HOMAS-IR.

Figure 8: Graphs of genes associated with insulin resistance in normal glucose tolerance (NGT), impaired glucose tolerance/ impaired fasting glucose (IGT/IFG) and type 2 diabetes (T2D), as measured by hyperinsulinemic euglycemic clamp.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The present invention provides for methods of profiling whole blood gene expression in a patient as a surrogate assessment of tissue low grade inflammation. The resulting data from this

method can be used as a complementary read-out of insulin resistance/associated co-morbidities for patient stratification and/or pharmacodynamic assessments. The methods find use in monitoring the effectiveness of an insulin sensitizer therapy or of an anti-diabetic therapy exerting insulin sensitizer effects given to a patient in need thereof. Examples of insulin sensitizers include, for example, metformin and thiazolidinediones, such as glitazones (troglitazone, rosiglitazone and pioglitazone).

The methods can also be applied to animal models (e.g. mice, rats, non-human primates) and provide a translational tool to bridge pre-clinical to clinical studies. As used herein “patient” refers to any mammal and includes, but is not limited to, humans, non-human primates, bovines, equines, canines, ovines, felines, and rodents. In one embodiment, the patient is a human. In one embodiment, the patient is a non-human primate.

In some embodiment, the patient is suffering from obesity, type 2 diabetes, insulin resistance, or other metabolic based condition such as NASH, or NAFLD, or is suspected of suffering from these conditions, or is predisposed to suffer from these conditions

Provided herein are gene or sets of genes (also referred to herein as biomarkers) to detect the presence of low grade inflammation, to assess patient sensitivity to or resistance to insulin and insulin sensitizers, and to monitor the effectiveness of a treatment or therapy on the metabolic conditions. The gene or gene set can also be used to detect or analyze the presence or prevalence of metabolic conditions such as type 2 diabetes, obesity, insulin resistant states, nonalcoholic steatohepatitis (NASH), and nonalcoholic fatty liver disease (NAFLD).

Gene expression profiles can also be used in the methods described herein. An expression profile or gene expression profile refers to the profile generated from the expression levels determined for each gene from the set of genes. Gene expression profiles are useful in monitoring and comparing changes over a set of genes. Gene expression profiles can be used, for example, to detect the presence of low grade inflammation, for assessing patient sensitivity to or resistance to insulin and insulin sensitizers, to determine the presence or prevalence of a metabolic condition, or to monitor the effectiveness of a treatment or therapy on the metabolic condition.

In one embodiment, gene expression profiles can be used to monitor the effectiveness of a therapy to treat a metabolic condition by determining the expression profile of a set genes in a patient undergoing therapy and comparing that expression profile to the expression profile of the same set of genes from a reference sample taken from the patient prior to therapy. The change in expression profile can then be compared to a control expression profile generated using the same set of genes from an individual, or individuals, not suffering from the metabolic condition. An effective treatment is indicated by a post-therapy gene expression profile that is more similar than the expression profile

of the sample taken from the patient prior to therapy to the expression profile of the control sample. An ineffective treatment is indicated by a gene expression profile that is the same as the gene expression profile of the patient prior to therapy or that is less similar than the expression profile of the sample taken from the patient prior to therapy to the expression profile of the control sample.

A control sample as used herein refers to any sample, standard, or level that is used for comparison purposes. In one embodiment, a control sample is obtained from a healthy individual who is not the patient. In certain embodiments, a control sample is a single sample or combined multiple samples from one or more healthy individuals who are not the patient. In certain embodiments, a control sample is a single sample or combined multiple samples from one or more individuals suffering from a metabolic disorder. In one embodiment, the control sample is a whole blood sample taken from one or more healthy individuals. In some embodiments, a healthy individual, or individuals, is not suffering from the metabolic disorder that is present in the patient, or is suspected of being present in the patient. In one embodiment, the healthy individual, or individuals, is not suffering from obesity, type 2 diabetes, insulin resistance, or other metabolic based condition such as NASH, or NAFLD. For example, a non-insulin resistant control sample is a sample that possesses the gene expression profile of a non-insulin resistant patient and can be generated, for example, by determining the gene expression levels of the set of genes used in the method from whole blood taken from a patient that is not suffering from obesity, type 2 diabetes, insulin resistance, or other metabolic based condition such as NASH, or NAFLD.

Genes useful in practicing the invention include the genes of Table 1. In one embodiment, the set of genes used in the methods described herein comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table 1.

One aspect of the invention provides for a method for detecting the presence of low grade inflammation in a patient comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient. In one embodiment, a change in expression profile of the set of genes as compared to a control sample of whole blood taken from a healthy individual indicates the presence of low grade inflammation in the patient. In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table

1.

In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of a set of genes comprising one or more genes that show a correlation between whole blood and adipose tissue expression, such as resistin, leptin, FoxP3, CD79A and CTLA4. In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of one, two, three, four, or all five of resistin, leptin, FoxP3, CD79A and CTLA4.

In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of a set of genes comprising one or more genes that show an association with insulin resistance, such as IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of one, two, three, or all four of IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, insulin resistance is determined by hyperinsulinemic euglycemic clamp or by homeostasis model assessment as an index of insulin resistance (HOMA-IR).

In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of a set of genes comprising one or more genes that show an association with elevated BMI, such as CEACAM8, RESISTIN, TNF α , IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NF κ B, CD36 and ICOS. In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or all thirteen of CEACAM8, RESISTIN, TNF α , IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NF κ B, CD36 and ICOS.

In one embodiment, the method for detecting the presence of low grade inflammation in a patient comprises determining the expression profile of a set of genes comprising one or more genes of Table 1 that are related to inflammation and NF κ B pathway.

In the above methods, a change in expression profile of the set of genes in the patient's sample as compared to a control sample indicates the presence of low grade inflammation in the patient.

The patient is suffering from obesity, type 2 diabetes, insulin resistance, or other metabolic based condition such as NASH, or NAFLD, or is suspected of suffering from these conditions, or is predisposed to suffer from these conditions.

Another aspect of the invention provides for a method of identifying a patient who may benefit from treatment with an insulin sensitizer comprising determining the expression profile of a set

of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the set of genes as compared to a control sample indicates that the patient may benefit from treatment with an insulin sensitizer. In one embodiment, the control sample is a whole blood sample taken from a patient who is not insulin resistant. In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table 1.

In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression level of a set of genes comprising one or more genes that show a correlation between whole blood and adipose tissue expression, such as resistin, leptin, FoxP3, CD79A and CTLA4. In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression level of one, two, three, four, or all five of resistin, leptin, FoxP3, CD79A and CTLA4.

In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression profile of a set of genes comprising one or more genes that show an association with insulin resistance, such as IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression profile of one, two, three, or all four of IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, insulin resistance is determined by hyperinsulinemic euglycemic clamp or by homeostasis model assessment as an index of insulin resistance (HOMA-IR).

In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression profile of a set of genes comprising one or more genes that show an association with elevated BMI, such as CEACAM8, RESISTIN, TNF α , IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NF κ B, CD36 and ICOS. In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression profile of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or all thirteen of CEACAM8, RESISTIN, TNF α , IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NF κ B, CD36 and ICOS.

In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer determining the expression level of a set of genes comprising one or more genes

of Table 1 that are related to inflammation and NFkB pathway.

In the above methods, a change in expression profile of the set of genes in the patient's sample as compared to a control sample indicates that the patient may benefit from treatment with an insulin sensitizer.

In one embodiment, the patient who is identified as one who may benefit from treatment with an insulin sensitizer is administered the insulin sensitizer subsequent to this analysis. In one embodiment, the methods herein are combined with other clinical parameters commonly used to select patients for treatment with an insulin sensitizer. These clinical parameters include, for example, HbA1c and plasma glucose (see as a reference Position Statement: Standards of Medical Care in Diabetes—2010, American Diabetes Association).

Yet another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising determining the expression profile of one or more genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the one or more genes as compared to a control sample indicates that the insulin sensitizer treatment is not effective. In one embodiment, the control sample is a whole blood sample taken from a patient that is not insulin resistant. In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table 1.

In one embodiment, the method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprises determining the expression profile of a set of genes comprising one or more genes that show a correlation between whole blood and adipose tissue expression, such as resistin, leptin, FoxP3, CD79A and CTLA4. In one embodiment, the method of identifying a patient who may benefit from treatment with an insulin sensitizer comprises determining the expression profile of one, two, three, four, or all five of resistin, leptin, FoxP3, CD79A and CTLA4

In one embodiment, the method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprises determining the expression profile of a set of genes comprising one or more genes that show an association with insulin resistance, such as IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, the method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprises determining the expression profile of one, two, three, or all four of IL1R1, CD36, TNFRSF10, and ICOS. In one embodiment, insulin resistance is determined by

hyperinsulinemic euglycemic clamp. In one embodiment, insulin resistance is determined by homeostasis model assessment as an index of insulin resistance (HOMA-IR).

In one embodiment, the method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprises determining the expression profile of a set of genes comprising one or more genes that show an association with elevated BMI, such as CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS. In one embodiment, the method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprises determining the expression profile of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or all thirteen of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS.

In one embodiment, the method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprises determining the expression level of a set of genes comprising one or more genes of Table 1 that are related to inflammation and NFkB pathway.

In the above methods, a change in expression profile of the set of genes in the patient's sample as compared to a control sample indicates that the effectiveness of the insulin sensitizer therapy.

In one embodiment, the insulin sensitizer therapy is altered or discontinued based on this analysis.

Another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising the steps of a) determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, b) comparing the expression profile of the set of genes to the expression profile of the set of genes from reference sample of whole blood taken from the patient prior to treatment with the insulin sensitizer, and c) determining that the therapy is effective when the expression profile of the genes in the test sample is more similar than the expression profile of the genes in the reference sample to the expression profile of a non-insulin resistant control sample. Conversely, the therapy is determined to be ineffective when the expression profile of the genes in the test sample is the same as the expression profile of the reference sample or is less similar than the expression profile of the genes in the reference sample to the expression profile of a non-insulin resistant control sample. If the therapy is ineffective, the treatment may be altered, for example, a different dose or dosing schedule of the same insulin sensitizer may be administered and similarly monitored for effectiveness or a different insulin sensitizer may be used in the therapy. In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-

two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, or all forty-two of the genes of Table 1.

Another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising the steps of a) determining the expression profile of a set of genes selected from the group consisting of resistin, leptin, FoxP3, CD79A and CTLA4 in a test sample of whole blood taken from the patient, b) comparing the expression profile of the set of genes to the expression profile of the set of genes from reference sample of whole blood taken from the patient prior to treatment with the insulin sensitizer, and c) determining that the therapy is effective when the expression profile of the genes in the test sample is more similar than the expression profile of the genes in the reference sample to the expression profile of non-insulin resistant control sample. Conversely, the therapy is determined to be ineffective when the expression profile of the genes in the test sample is the same as the expression profile of the reference sample or is less similar than the expression profile of the genes in the reference sample to the expression profile of a non-insulin resistant control sample. If the therapy is ineffective, the treatment may be altered, for example, a different dose or dosing schedule of the same insulin sensitizer may be administered and similarly monitored for effectiveness or a different insulin sensitizer may be used in the therapy. In one embodiment, the set of genes comprises one, two, three, four, or all five of the genes selected from among resistin, leptin, FoxP3, CD79A and CTLA4.

Another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising the steps of a) determining the expression profile of a set of genes selected from the group consisting of IL1R1, CD36, TNFRSF10, and ICOS in a test sample of whole blood taken from the patient, b) comparing the expression profile of the set of genes to the expression profile of the set of genes from reference sample of whole blood taken from the patient prior to treatment with the insulin sensitizer, and c) determining that the therapy is effective when the expression profile of the genes in the test sample is more similar than the expression profile of the genes in the reference sample to the expression profile of non-insulin resistant control sample. Conversely, the therapy is determined to be ineffective when the expression profile of the genes in the test sample is the same as the expression profile of the reference sample or is less similar than the expression profile of the genes in the reference sample to the expression profile of a non-insulin resistant control sample. If the therapy is ineffective, the treatment may be altered, for example, a different dose or dosing schedule of the same insulin sensitizer may be administered and similarly monitored for effectiveness or a different insulin sensitizer may be used in the therapy. In one embodiment, the set of genes comprises one, two, three, or all four of the genes selected from

IL1R1, CD36, TNFRSF10, and ICOS.

Another aspect of the invention provides for a method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising the steps of a) determining the expression profile of a set of genes selected from the group consisting of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS in a test sample of whole blood taken from the patient, b) comparing the expression profile of the set of genes to the expression profile of the set of genes from reference sample of whole blood taken from the patient prior to treatment with the insulin sensitizer, and c) determining that the therapy is effective when the expression profile of the genes in the test sample is more similar than the expression profile of the genes in the reference sample to the expression profile of non-insulin resistant control sample. Conversely, the therapy is determined to be ineffective when the expression profile of the genes in the test sample is the same as the expression profile of the reference sample or is less similar than the expression profile of the genes in the reference sample to the expression profile of a non-insulin resistant control sample. If the therapy is ineffective, the treatment may be altered, for example, a different dose or dosing schedule of the same insulin sensitizer may be administered and similarly monitored for effectiveness or a different insulin sensitizer may be used in the therapy. In one embodiment, the set of genes comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or all thirteen of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS.

Another aspect of the invention provides for the use of a gene or set of genes selected from the group consisting of the genes of Table 1 to determine the presence of low grade inflammation in a patient, to identify a patient who may benefit from treatment with an insulin sensitizer, or to monitor the effectiveness of an insulin sensitizer therapy given to a patient. In one embodiment, the expression level of the gene or expression profile of a set of genes is determined.

Using sequence information provided by the database entries for the known sequences or the chip manufacturer, sequences can be detected (if expressed) and measured using techniques well known to one of ordinary skill in the art. Expression levels/amount of a gene or a biomarker can be determined based on any suitable criterion known in the art, including but not limited to mRNA, cDNA, proteins, protein fragments and/or gene copy number.

Expression of various genes or biomarkers in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including but not limited to, immunohistochemical and/or Western blot analysis, immunoprecipitation, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting (FACS) and the like,

quantitative blood based assays (as for example Serum ELISA) (to examine, for example, levels of protein expression), biochemical enzymatic activity assays, in situ hybridization, Northern analysis and/or PCR analysis of mRNAs, as well as any one of the wide variety of assays that can be performed by gene and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel et al. eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis). Multiplexed immunoassays such as those available from Rules Based Medicine or Meso Scale Discovery (MSD) may also be used.

In certain embodiments, expression/amount of a gene or biomarker in a sample is increased as compared to expression/amount in a reference or control sample if the expression level/amount of the gene or biomarker in the sample is greater than the expression level/amount of the gene or biomarker in the reference or control sample. Similarly, expression/amount of a gene or biomarker in a sample is decreased as compared to expression/amount in a reference or control sample if the expression level/amount of the gene or biomarker in the sample is less than the expression level/amount of the gene or biomarker in the reference or control sample. In one embodiment, the expression level is mRNA expression level. In one embodiment, the change in mRNA expression level is an increase. In another embodiment, the change in the mRNA expression level is a decrease. In certain embodiments, the samples are normalized for both differences in the amount of RNA or protein assayed and variability in the quality of the RNA or protein samples used, and variability between assay runs. Such normalization may be accomplished by measuring and incorporating the expression of certain normalizing genes, including well known housekeeping genes, such as GAPDH or β actin. Alternatively, normalization can be based on the mean or median signal of all of the assayed genes or a large subset thereof (global normalization approach). On a gene-by-gene basis, measured normalized amount of a patient tumor mRNA or protein is compared to the amount found in a reference or control set. Normalized expression levels for each mRNA or protein per tested tumor per patient can be expressed as a percentage of the expression level measured in the reference or control set. The expression level measured in a particular patient sample to be analyzed will fall at some percentile within this range, which can be determined by methods well known in the art.

In certain embodiments, the expression level of a gene or biomarker in a test sample can be considered changed if its expression level changes (either increases or decreases) by about 1.5 fold, 2 fold, 3 fold, 5 fold, 10 fold, or more from the expression level of the corresponding gene or biomarker in the reference or control sample. In certain embodiments, the expression level of a gene or biomarker in a test sample can be considered changed if its expression level changes (either increases or decreases) by about 50%, 75%, 100% 150%, 200%, 500% or more from the expression level of the

corresponding gene or biomarker in the reference or control sample. In one embodiment, the expression level is mRNA expression level. In one embodiment, the expression level is determined based on protein expression level.

Methods of the invention further include protocols which examine the presence and/or expression level of mRNAs of the one or more target genes in a tissue or cell sample.

Methods for the evaluation of mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as in situ hybridization using labeled riboprobes) specific for the one or more genes, including, but not limited to, the genes of Table 1, Northern blot and related techniques and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for one or more of the genes, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like).

In one embodiment, the sample is a whole blood sample. In another embodiment, the sample is peripheral blood mononuclear cells (PBMCs).

Samples from patients can be conveniently assayed for mRNAs using Northern, dot blot or PCR analysis. For example, RT-PCR assays such as quantitative PCR assays are well known in the art. In an illustrative embodiment of the invention, a method for detecting a target mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using a target polynucleotide as sense and antisense primers to amplify target cDNAs therein; and detecting the presence of the amplified target cDNA. In addition, such methods can include one or more steps that allow one to determine the levels of target mRNA in a biological sample (e.g., by simultaneously examining the levels a comparative control mRNA sequence of a "housekeeping" gene such as an actin family member). Optionally, the sequence of the amplified target cDNA can be determined.

Optional methods of the invention include protocols which examine or detect mRNAs, such as target mRNAs, in a sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes whose expression correlate with detection of inflammation may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. Differential gene expression analysis of disease tissue can provide valuable information. Microarray technology utilizes nucleic acid hybridization techniques and computing technology to evaluate the

mRNA expression profile of thousands of genes within a single experiment (see, e.g., WO 01/75166 published October 11, 2001; (see, for example, U.S. 5,700,637, U.S. Patent 5,445,934, and U.S. Patent 5,807,522, Lockart, *Nature Biotechnology*, 14:1675-1680 (1996); Cheung, V.G. et al, *Nature Genetics* 21(Suppl):15-19 (1999) for a discussion of array fabrication). DNA microarrays are miniature arrays containing gene fragments that are either synthesized directly onto or spotted onto glass or other substrates. Thousands of genes are usually represented in a single array. A typical microarray experiment involves the following steps: 1) preparation of fluorescently labeled target from RNA isolated from the sample, 2) hybridization of the labeled target to the microarray, 3) washing, staining, and scanning of the array, 4) analysis of the scanned image and 5) generation of gene expression profiles. Currently two main types of DNA microarrays are being used: oligonucleotide (usually 25 to 70 mers) arrays and gene expression arrays containing PCR products prepared from cDNAs. In forming an array, oligonucleotides can be either prefabricated and spotted to the surface or directly synthesized on to the surface (in situ).

The Affymetrix GeneChip® system is a commercially available microarray system which comprises arrays fabricated by direct synthesis of oligonucleotides on a glass surface.

Probe/Gene Arrays: Oligonucleotides, usually 25 mers, are directly synthesized onto a glass wafer by a combination of semiconductor-based photolithography and solid phase chemical synthesis technologies. Each array contains up to 400,000 different oligos and each oligo is present in millions of copies. Since oligonucleotide probes are synthesized in known locations on the array, the hybridization patterns and signal intensities can be interpreted in terms of gene identity and relative expression levels by the Affymetrix Microarray Suite software. Each gene is represented on the array by a series of different oligonucleotide probes. Each probe pair consists of a perfect match oligonucleotide and a mismatch oligonucleotide. The perfect match probe has a sequence exactly complementary to the particular gene and thus measures the expression of the gene.

The mismatch probe differs from the perfect match probe by a single base substitution at the center base position, disturbing the binding of the target gene transcript. This helps to determine the background and nonspecific hybridization that contributes to the signal measured for the perfect match oligo. The Microarray Suite software subtracts the hybridization intensities of the mismatch probes from those of the perfect match probes to determine the absolute or specific intensity value for each probe set. Probes are chosen based on current information from Genbank and other nucleotide repositories. The sequences are believed to recognize unique regions of the 3' end of the gene. A GeneChip Hybridization Oven ("rotisserie" oven) is used to carry out the hybridization of up to 64 arrays at one time.

The fluidics station performs washing and staining of the probe arrays. It is completely automated and contains four modules, with each module holding one probe array. Each module is controlled independently through Microarray Suite software using preprogrammed fluidics protocols. The scanner is a confocal laser fluorescence scanner which measures fluorescence intensity emitted by the labeled cRNA bound to the probe arrays. The computer workstation with Microarray Suite software controls the fluidics station and the scanner. Microarray Suite software can control up to eight fluidics stations using preprogrammed hybridization, wash, and stain protocols for the probe array. The software also acquires and converts hybridization intensity data into a presence/absence call for each gene using appropriate algorithms. Finally, the software detects changes in gene expression between experiments by comparison analysis and formats the output into .txt files, which can be used with other software programs for further data analysis.

Expression of a selected gene or biomarker in a tissue or cell sample may also be examined by way of functional or activity-based assays. For instance, if the biomarker is an enzyme, one may conduct assays known in the art to determine or detect the presence of the given enzymatic activity in the tissue or cell sample.

Also provided are kits comprising a compound capable of specifically detecting expression levels of the genes of Table 1, wherein the kit further comprises instructions for using the kit to determine the presence of low grade inflammation in a patient or to predict or monitor responsiveness of a patient to insulin sensitizer therapy.

One embodiment provides for a kit comprising a container, a label on the container, and a composition contained within the container; wherein the composition includes one or more polynucleotides that specifically hybridize to a gene of Table I, the label on the container indicates that the composition can be used to evaluate the presence of a gene of Table I in a sample, and instructions for using the polynucleotide for evaluating the presence of a gene of Table I in the sample. In one embodiment, the sample is a whole blood sample, or derived from a whole blood sample.

Other optional components in the kit include one or more buffers (e.g., block buffer, wash buffer, substrate buffer, etc), other reagents such as substrate (e.g., chromogen) which is chemically altered by an enzymatic label, epitope retrieval solution, control samples (positive and/or negative controls), control slide(s) etc.

Examples

Example 1 - Listing of Genes useful in practicing the invention

Table 1 provides a list of genes useful for practicing the invention, including for use in

detection of the presence of low grade inflammation, for assessing patient sensitivity to or resistance to insulin and insulin sensitizers, and for monitoring the effectiveness of a treatment or therapy on the metabolic conditions. Also included in Table 1 are the Gene IDs associated with the listed genes, as well as the sequence listing identifiers referring to an exemplary sequence of the gene in the Sequence Listing provided herein.

Table 1

Gene name	Gene ID	SEQ ID NO:	TaqMan Assay ID
RELB	5971	1	Hs00232399_m1
SLC20A1	6574	2	Hs00965596_m1
TGFB1	7040	3	Hs00998133_m1
MIF	4282	4	Hs00236988_g1
CCL2 (MCP-1)	6347	5	Hs00234140_m1
MMP9	4318	6	Hs00957562_m1
visfatin* (NAMPT)	10135	7	Hs00237184_m1
Resistin (RETN)	56729	8	Hs00220767_m1
ADIPOR1	51094	9	Hs01114951_m1
ADIPOR2	79602	10	Hs00226105_m1
CMKLR1	1240	11	Hs01386064_m1
RBP4	5950	12	Hs00198830_m1
CEACAM8 (CD66b)	1088	13	Hs00266198_m1
CD16 (FCGR3B)	2215	14	Hs00275547_m1
BLK	640	15	Hs01017452_m1
MS4A1 (CD20)	931	16	Hs00174849_m1
CD79A	973	17	Hs00233566_m1
CD90 (THY)	7070	18	Hs00174816_m1
ICOS	29851	19	Hs00359999_m1
CTLA4	1493	20	Hs03044418_m1
CD14	929	21	Hs02621496_s1
CSF1R	1436	22	Hs00911250_m1
MARCO	8685	23	Hs00198937_m1
FCGR3A (CD16A)	2214	24	Hs02388314_m1
TNFRSF10C	8794	25	Hs00921974_m1
VNN2	8875	26	Hs00190581_m1
IL6	3569	27	Hs00985639_m1
IL1R1	3554	28	Hs00168392_m1
NFKB1	4790	29	Hs00765730_m1
AGER	177	30	Hs00965859_g1
TLR4	7099	31	Hs00152939_m1
TNFalpha	7124	32	Hs00174128_m1
TNFRSF1A	7132	33	Hs01042313_m1

TNFRSF1B	7133	34	Hs00153550_m1
ADIPOQ (Adiponectin)	9370	35	Hs00605917_m1*
FABP4	2167	36	Hs01086177_m1*
IFNG	3458	37	Hs00989291_m1*
RARRES2	5919	38	Hs01123775_m1*
CD36	948	39	Hs00169627_m1
FoxP3	50943	40	Hs01085834_m1
PPIB	5479	41	Hs01018503_m1
GUSB	2990	42	Hs99999908_m1

Example 2 -Analysis of Whole Blood and PBMCs

A panel of genes related to key nodes of inflammation pathways, and a panel of genes specific for each blood cell type present in whole blood preparations were measured in a cross-sectional sample collections of lean and obese and insulin resistant (IR) subjects. Table 1. The total sample size was 40 with 20 obese and 20 normal weight subjects. Blood-cell specific genes were measured as a means of performing an indirect assessment of the different population's enrichment and/or activation status in samples from obese and lean subjects.

Quantitative Real-time PCR

Samples from both PBMC and whole blood were analyzed using quantitative real-time PCR. RNA extraction was done from the PAXgene blood on a BioRobot MDx following the manufacturer's protocol (Qiagen) while RNA extraction from PBMC samples were performed using Qiagen QIAcube using the QIAamp RNA Blood kit (Qiagen, Germany) RNA was quantified with the Quant-IT RiboGreen kit (Molecular Probes, Invitrogen) at 50-fold dilution against standard curves of Escherichia coli ribosomal RNA (Roche Diagnostics). In addition, samples were also analyzed for RNA integrity number (RIN) on the Agilent Bioanalyzer according to manufacture's recommended protocol (Agilent)

cDNA synthesis was done with the SuperScript II First- Strand Synthesis SuperMix for quantitative real-time PCR (qRT-PCR; Invitrogen) on 400 ng total RNA following the manufacturer's protocol but with omission of the RNase H digest. For each reverse transcription reaction, Universal Human Reference total RNA (Stratagene) was run as a positive and negative control (nonenzyme control) on the same plate. Controls were assayed by qRT-PCR. Predesigned gene expression assays were obtained from Applied Biosystems. The TaqMan Assay IDs are shown in Table 1.

Where possible, exon-spanning assays were selected to ensure cDNA specificity. All gene

expression assays were done on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) with the recommended standard settings. All assays were prepared with the TaqMan Universal PCR Master Mix (Applied Biosystems) following the manufacturer's recommendations.

All runs included a standard curve dilution of cDNA, a nonenzyme control, a nontemplate control, and a calibrator sample. Standard curve cDNA was synthesized from Universal Human Reference total RNA (Stratagene) and the calibrator sample from a pool of total blood RNA from healthy donors. cDNA samples were diluted 10-fold in molecular-grade water, and 2 uL were added to 18 uL predistributed assay Master Mix. This corresponds to cDNA from 4 ng total RNA. All samples on a plate were assayed with one assay for a gene of interest and the endogenous control gene assays, GUSB and PPIB TaqMan Gene Expression Assays; Applied Biosystems). Each measurement was done in triplicate.

Example 3 - Statistical Analysis of qRT-PCR Data

For each target, the median Ct was calculated. Δ Ct values were calculated with the following equation: (Δ Ct = (Target gene median Ct value) - (Geometric mean of GUSB and PPIP)).

Expression values (Δ CT) were multiplied by -1 so that higher values represent greater gene expression. Forward selection was used to enter the other clinical covariates into the model, one at a time, and significant ($p < 0.05$) covariates were retained. A complete-case analysis was performed. PBMC and whole blood samples were analysed separately.

The data were therefore modeled as

$$y_i = \beta_0 + \beta_1 \text{BMI}_i + \beta_2 \text{age}_i + \beta_3 \text{sex} + \epsilon_i$$

where • y_i = gene expression for individual i ; • β_0 = intercept; • β_1 = effect of BMI; • β_2 = effect of age; • β_3 = effect of sex (0 = female, 1 = male); and • $\epsilon_i \sim N(0, \sigma^2)$.

Example 4- Whole blood gene expression analysis detects low grade inflammation in obese patients

As shown in Figure 1, obese (body mass index (BMI) of > 30) and lean ($\text{BMI} < 25$) subjects cluster apart from each other based on gene expression patterns (descriptive statistical analysis). In addition, as shown in Figure 2 some of the gene analysed show a positive correlation to BMI (e.g. IL6, TNFa, Resistin, MIF), whereas some others show a negative correlation (e.g. IL1R1, TNFRSF1A, TLR4). In details, as shown in Figure 3 (showing Δ CT of the genes for lean and obese patients), out

of the 33 genes analysed, some are upregulated in the whole blood of obese subjects as compared to lean (IL6, TNFa, CEACAM8, Resistin), whereas some others are downregulated in the whole blood of obese subjects as compared to lean (e.g. TNFRSF1A, TNFRSF1B, IL1R1, TLR4). Since some of these gene are mapped on NFkB pathway (upstream NFkB: TNFRSFs, IL1R1, TLR4, downstream NFkB: IL6 and TNFa), these results support a differential regulation of NFkB pathway in whole blood from obese subjects as compared to lean. While the upregulation of IL6 and TNFa is in line with previous findings and clearly indicates an upregulation of the NFkB activity, as known in pro-inflammatory states, it is not clear why the genes encoding for cell-membrane receptor directly facing the pro-inflammatory environment (e.g. TNFRSF1A, B, TLR4, IL1R1) are down-regulated. It could be that gene expression reflects a regulatory feedback mechanisms whereby the genes are downregulated in response to an higher expression/activity of the encoded proteins on the cell membrane. In addition, some genes expressed in granulocytes and activated monocytes, such as CEACAM8 and Resistin, are upregulated in obese subjects, which supports an activation of the innate immune system in these subjects. Interestingly, based on the gene expression levels of the blood-cell specific genes, there is no evidence for an overall change in cells abundance (Figure 4), therefore the changes in the genes upstream and downstream NFkB node seem to be truly linked to a differential regulation of the pathway corresponding to a pro-inflammatory state.

This study shows that gene expression in whole blood reflects the activation of key pathways shown to be upregulated also in adipose tissue, such as NFkB, thus supporting the use of whole blood gene expression as a surrogate of tissue gene expression.

These findings are in agreement with previous reports (1,2) on gene expression of some of the noted genes in PBMC and flow-cytometry analyses in whole blood. Additionally, this study provides additional genes of relevance to the detection of inflammation, such as CEACAM8.

As shown in the Examples, a gene expression analysis for the same targets was also conducted in matched PBMC samples for comparison. As expected, PBMC showed overall a different expression profile as compared to whole blood. As shown in Figure 5, whole blood shows an enrichment of granulocyte-specific genes (e.g. FCGR3B, TNFRSF10C, VNN2, CEACAM8, CD16) as compared to PBMC (left part of the graph, values below 0), whereas PBMC show an enrichment in monocytes-specific genes (e.g. CSF1R and MARCO). The genes found to be differentially regulated in whole blood between lean and obese show only a modest trend in the matched PBMC samples (data not shown). This could be explained by a greater contribution of granulocytes to overall inflammatory state.

In summary, these results indicate that (i) whole blood gene expression could be used in

clinical settings as a tool to assess low grade inflammation state for patient stratification and/or for pharmacodynamic assessments (ii) in the method could also be applied to animal models (e.g. non-human primates) and could provide a translational tool to bridge pre-clinical to clinical studies.

Example 5 – Whole blood gene expression as a surrogate of adipose tissue inflammation

A set of genes related to insulin resistance (resistin), leptin resistance (leptin) and T-cell (CD79A), T reg cells (FoxP3) and B-cell mediated inflammation (CTLA4) is correlated in adipose tissue and in whole blood. Involvement of B cells, T cells and T reg cells in adipose inflammation is supported by previous evidences (15, 16, 17). The finding that markers of those cells are regulated in the same manner in whole blood supports the concept that whole blood can be used a surrogate matrix for assessment of tissue inflammation. Figure 6.

Example 6 –Expression of genes associated with type 2 diabetes

Homeostatic Model Assessment (HOMA-IR) was used to determine gene expression associated with type 2 diabetes. HOMA-IR index was calculated as follows (18): Fasting insulin x Fasting glucose = 22.5. Figure 7 shows a scatterplot representing the association between the expression (ΔCt) of three of the genes of the panel (IL1R1, TNFRSF10C, ICOS) and an index of insulin sensitivity (HOMAS or HOMA-IR) in a type 2 diabetes population (n=87, mean HbA1c: 7.8%)

Example 7 –Expression of genes associated with IR in NGT, IGT/IFG, and T2D

A hyperinsulinemic euglycemic clamp assay was used to determine gene expression associated with insulin resistance in normal glucose tolerance (NGT), impaired glucose tolerance/ impaired fasting glucose (IGT/IFG) and type 2 diabetes (T2D).

The hyperinsulinemic euglycemic clamp (HEC) was conducted based on the principles established by DeFronzo 1979 (19) and Ferranini 1998 (20). Briefly, an intravenous catheter was inserted into an antecubital vein of one arm for the infusion of insulin and a 20% glucose solution (Insulin Humulin R, 100 m.j./ml, Lilly; glucose 20% intravenous infusion B.P. Bieffe, composition: Glucosum monohydricum 220 g = 200 g Glucosum/1000 ml). A second cannula was inserted into a dorsal hand vein, warmed at about 70°C for the sampling at 5 min intervals of arterialised blood. The glucose infusion rate was adjusted according to the changes in blood glucose concentration. The continuous rate of insulin infusion was 1 mIU/kg/min for patients with a BMI < 30 kg/m², and 40 mIU/m²/min for patients with a BMI ≥ 30 kg/m². During the first 10 min, the rate of infusion was doubled for faster insulin loading. The insulin infusion was maintained for 180 min, with steady state

period lasting from 120 to 180 min. The two insulin sensitivity indexes M and ISI were derived as described below:

Calculation of M

(according to De Fronzo 1979 (19))

Glucose infusion rate **INF** (sometimes called GIR) was calculated as follows:

Glucose infusion rate **INF** (sometimes called GIR) was calculated as follows:

$$\text{INF} = \frac{\text{Average infusion rate in steady state ml/60 min} * \text{infusate concentration mg/ml}}{\text{Patient weight kg}} = \frac{\text{mg}}{\text{min*kg}}$$

Space Correction **SC** was calculated as the difference between the Plasma Glucose concentration G1 at the beginning of the steady state period subtracted from the Plasma Glucose concentration G2 at the end of the steady state period, multiplied by 18 for correction of units (from mmol/L to mg/dL), again multiplied by the fraction of body glucose space, divided by the duration of the steady state period. (see De Fronzo 1979 for more explanation):

$$\text{SC} = \frac{(\text{G2} - \text{G1 Plasma Glucose mmol/L}) * 18 \text{ mg*L}/(\text{mmol*dL}) * 10 \text{ dL/L} * 0.19 \text{ L/kg}}{60 \text{ min}} = \frac{\text{mg}}{\text{min*kg}}$$

Calculation of Glucose Metabolized **M**:

$$\text{M} = \text{INF} - \text{SC} = \text{mg}/(\text{kg*min})$$

Calculation of Insulin Sensitivity Index

(according to Coates 1995 (21))

$\text{ISI} = \text{M}/(\text{G} \times \Delta\text{I})$, where M is the glucose metabolized at steady state, G is the steady state blood glucose concentration and ΔI is the difference between fasting and steady state plasma insulin concentration. For steady state blood glucose concentration, the average of the blood glucose value in mmol/L at 120 min and at 180 min was taken, and multiplied with 18 to convert to mg/dL. For ΔI , the basal Insulin concentration (average of the -5 min, 0 min, and pre-clamp value) was subtracted from the steady state plasma insulin concentration (average of the 120 min and the 180 min value), all in mIU/L. The result was multiplied by 10^4 to arrive at the Units $10^{-2} \cdot \text{I}^2 / (\text{IU} \cdot \text{min} \cdot \text{kg})$.

Figure 8 shows the resulting scatterplots representing the association between the expression (ΔCt) of some genes of the panel and two indexes of insulin sensitivity (ISI and M) obtained with the hyperinsulinemic euglycemic clamp. Out of the 10 genes analysed, only CD36 shows a consistent and significant association to both ISI and M, whereas TNF α only shows a trend for an association with ISI. Population analysed included normoglycemic (NGT), pre-diabetics (impaired glucose tolerant, IGT) and diabetic (T2D) individuals with the following characteristics:

Table 2 : Demographics and Baseline Characteristics

Mean values		NGT	IGT	T2D
Number of subjects	n	11	11	10
Female	n (%)	6 (55%)	6 (55%)	5 (50%)
Age	years	53.1	58.1	61.1
Weight	kg	84.1	86.4	84.3
Height	cm	171	169	168
BMI	kg/m ²	29.0	30.3	29.5
Waist:Hip Ratio	ratio	0.88	0.94	0.93

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference

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Claims

1. A method for detecting the presence of low grade inflammation in a patient comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the set of genes as compared to a healthy control sample indicates the presence of low grade inflammation in the patient.
2. A method of identifying a patient who may benefit from treatment with an insulin sensitizer comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the set of genes as compared to a non-insulin resistant control sample indicates that the patient may benefit from treatment with an insulin sensitizer.
3. A method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient, wherein a change in expression profile of the set of genes as compared to a non-insulin resistant control sample indicates that the insulin sensitizer treatment is not effective.
4. The method of any of the preceding claims, wherein the set of genes comprises one or more genes selected from the group consisting of resistin, leptin, FoxP3, CD79A and CTLA4.
5. The method of claim 4, wherein the set of genes comprises two, three, four, or all five of resistin, leptin, FoxP3, CD79A and CTLA4.
6. The method of any of the preceding claims, wherein the set of genes comprises one or more genes selected from the group consisting IL1R1, CD36, TNFRSF10, and ICOS.
7. The method of claim 7, wherein the set of genes comprises two, three, or all four of IL1R1, CD36, TNFRSF10, and ICOS.
8. The method of any of the preceding claims, wherein the set of genes comprises one or more genes selected from the group consisting of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS.
9. The method of claim 9, wherein the set of genes comprises two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or all thirteen of CEACAM8, RESISTIN, TNFa, IL6, ILR1, TLR4, TNFRSF1A, TNFRSF1B, MIF, CMKLR1, NFkB, CD36 and ICOS.
10. The method of any of the preceding claims, wherein the patient is suffering from a

metabolic condition.

11. The method of claim 10, wherein the metabolic condition is type 2 diabetes, obesity, insulin resistant states, nonalcoholic steatohepatitis (NASH), or nonalcoholic fatty liver disease (NAFLD).

12. The method of any of the preceding claims, wherein determining the expression profile comprises determining the mRNA expression level.

13. The method of claim 12, wherein the mRNA expression level is measured using qRT-PCR.

14. The method of any one of the preceding claims, wherein the change in expression level of the set of genes in the test sample is at least about a 1.5 fold difference as compared to the control sample.

15. A method of monitoring effectiveness of an insulin sensitizer therapy given to a patient comprising the steps of

a) determining the expression profile of a set of genes selected from the group consisting of the genes of Table 1 in a test sample of whole blood taken from the patient,

b) comparing the expression profile of the set of genes to the expression profile of the set of genes from reference sample of whole blood taken from the patient prior to treatment with the insulin sensitizer, and

c) determining that the therapy is effective when the expression profile of the genes in the test sample is more similar than the expression profile of the genes in the reference sample to the expression profile of non-insulin resistant control sample.

16. The methods substantially as hereinbefore described, especially with reference to the foregoing examples.