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(71) Applicant *(for all designated States except US)*: **QUANTOMIX LTD.** [IL/IL]; P.O. Box 4037, 70400 Nes-Ziona (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ZIK, Ory** [IL/US]; 15 Columbia St., Brookline, Massachusetts 02446 (US). **BEHAR, Vered** [IL/IL]; P.O. Box 15290, 90815 Moshav Bait Zait (IL).

(74) Agents: **PEARL COHEN ZEDEK LATZER** et al.; 5 Shenkar Street, 46733 Herzelia (IL).

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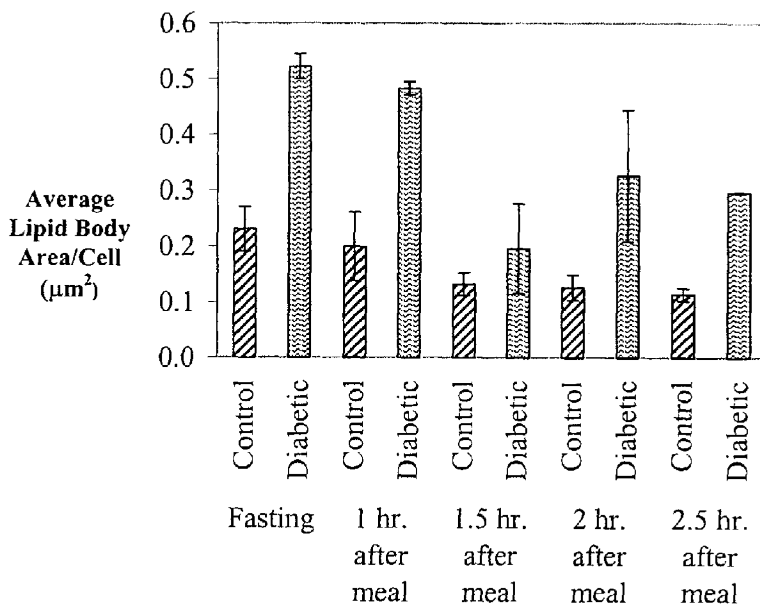
(54) **Title:** DEVICE AND METHOD FOR ANALYSIS OF A METABOLIC MALADY



(57) **Abstract:** A method for detecting a metabolic malady in a subject, including introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from the subject, and analyzing a quantitative feature of a lipid body in the leukocyte, wherein if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a threshold, the subject is detected as having a metabolic malady.

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DEVICE AND METHOD FOR ANALYSIS OF A METABOLIC MALADY

FIELD OF INVENTION

The present invention relates generally to methods for analyzing a metabolic
5 malady or other abnormality.

BACKGROUND

Metabolic maladies or abnormalities are among the most common chronic human diseases and complications. Metabolic maladies include, for example, without
10 limitation, metabolic diseases, metabolic disorders, atherosclerosis, glucose intolerance, type II diabetes, metabolic syndrome, insulin resistance, pre-diabetes, lipotoxicity, fatty liver, steatohepatitis, steatosis, obesity, stroke, cardio-vascular diseases, hyperlipidemia, or metabolic malady complications, such as diabetes complications, such as diabetic retinopathy or diabetic nephropathy or any other risk factor pertaining to metabolic
15 diseases or disorders. The etiology of the metabolic malady is considered to be multifactorial involving genetic and environmental effects.

Atherosclerosis is a coronary artery disease caused by fatty deposit build up in blood vessel walls that narrow the passageway for the movement of blood within blood vessels. This may lead to eventual blockage of the coronary arteries resulting in a heart
20 attack, the leading cause for premature death in the United States.

Fatty liver encompasses a spectrum of clinical conditions characterized histologically by mainly macrovesicular steatosis of the liver. The histopathological spectrum of fatty liver disease ranges from the simple fatty liver (steatosis) to the steatohepatitis, a variant, which has variable degrees of fibrosis. Steatohepatitis may be
25 progressive and can lead to cirrhosis, liver failure and hepatocellular carcinoma and may be a major cause of cryptogenic cirrhosis. The common risk factors for fatty liver disease are obesity, type II diabetes, and hyperlipidemia.

Type II diabetes is among the most common chronic human diseases, affecting almost 8% of the adult population and 19% of people above the age of 65 years in the
30 United States.

Metabolic syndrome is a cluster of risk factors for various diseases, such as cardio-vascular diseases and diabetes. For example, 25% of adults living in the United

States are diagnosed with metabolic syndrome. It is believed that the pathophysiology of the metabolic syndrome is related to insulin resistance. The risk factors may be generally defined as an accumulation of the following: elevated waist circumference, such as equal to or greater than 102 cm in man and 88 cm in women; elevated triglycerides, such as equal to or greater than 150 mg/dL; reduced high-density lipoprotein (HDL) cholesterol, such as less than 40 mg in men and 50 mg/dL in women; elevated blood pressure, such as equal to or greater than 130/85 mm Hg and elevated fasting glucose, such as equal to or greater than 100 mg/dL. It is appreciated that the metabolic syndrome may include other risk factors. Additionally, the risk factors may vary in different populations.

Many of the metabolic maladies are characterized by triglyceride accumulation and insulin resistance.

Triglyceride accumulation in various body tissues, such as muscle, liver and pancreas tissue is considered to be an important factor of organ specific insulin resistance leading to the development of a metabolic malady. Furthermore, accumulation of lipid droplets, which is identical to the term lipid bodies, in tissues occurs early in the development of insulin resistance and is correlated with its severity. A number of scientific articles describe a method for diagnosis of insulin-resistant related diseases by measuring lipid content in muscle tissue (Kelley DE, Goodpaster BH, and Storlien L. Muscle triglyceride and insulin resistance. Annu Rev Nutr 22: 325-346, 2002; Goodpaster B and Kelley D. Skeletal muscle triglyceride: marker or mediator of obesity-induced insulin resistance in type II diabetes mellitus. Current Diabetes Reports 2: 216-222, 2002).

SUMMARY

Embodiments of the invention may provide a method for diagnosing, detecting, predicting and/or monitoring the presence and severity of a metabolic malady, as well as a method for screening the efficiency and/or efficacy of ligands for treating or preventing the same.

There is thus provided in accordance with an embodiment of the present invention

a method including introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from a subject, and analyzing a quantitative feature of a lipid body in the leukocyte.

In accordance with an embodiment of the present invention a method for
5 metrology of a quantitative feature of a lipid body in a leukocyte sample includes introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from a subject, and analyzing the quantitative feature of the lipid body in the leukocyte.

There is thus provided in accordance with an embodiment of the present invention
10 a method for detecting a metabolic malady in a subject, including introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from the subject, and analyzing a quantitative feature of a lipid body in the leukocyte, wherein if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a
15 threshold, the subject may be detected as having a metabolic malady.

In accordance with an embodiment of the present invention a method for early detection of a metabolic malady includes introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from a subject, and analyzing a quantitative feature of a lipid body in the leukocyte, wherein if a value
20 of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a threshold, the subject may be detected as having a susceptibility for developing a metabolic malady.

There is thus provided in accordance with an embodiment of the present invention a method for monitoring a metabolic malady including introducing a blood
25 sample containing a leukocyte into an examination system, the blood sample being derived from a subject, and analyzing a quantitative feature of a lipid body in the leukocyte by evaluating if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a threshold, thereby monitoring a metabolic malady.

30 In accordance with an embodiment of the present invention a method for assessing the efficacy of a ligand for metabolic malady prevention or treatment includes obtaining a blood sample containing a leukocyte from a subject with a metabolic

malady, following administration of the ligand to the subject, introducing the blood sample into an examination system, and analyzing a quantitative feature of a lipid body in the leukocyte of the blood sample, wherein if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample is lower than a value of a quantitative feature of a lipid body in a leukocyte of a blood sample of an untreated subject with a metabolic malady, the ligand may be detected as efficacious for treatment or prevention of a metabolic malady.

There is thus provided in accordance with an embodiment of the present invention a kit for metabolic malady analysis of a subject, the kit including a sample container for inserting a blood sample containing a leukocyte therein, and data for analysis of a quantitative feature of a lipid body in the leukocyte so as to detect the presence of the metabolic malady.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C and 1D are each a simplified flow chart of a method in accordance with an embodiment of the present invention;

Figures 2A and 2B are each a simplified pictorial illustration of a kit constructed and operative in accordance with an embodiment of the present invention;

Figure 3 (A and B) is a micrograph of a neutrophil-containing sample of a representative healthy human subject (A) and a representative metabolic syndrome patient (B);

Figure 4 (A and B) is a micrograph of a monocyte-containing sample of a representative healthy human subject (A) and a representative metabolic syndrome patient (B);

Figure 5 is a graph of the average number of lipid bodies per neutrophil cell of leukocyte samples obtained from two healthy human subjects and two metabolic syndrome patients following a 12 hour fast;

Figure 6 is a graph of the average lipid body area per neutrophil cell of leukocyte samples obtained from two healthy human subjects and two metabolic syndrome patients following a 12 hour fast;

Figure 7 is a graph of the average number of lipid bodies per monocyte cell of leukocyte samples obtained from two healthy human subjects and two metabolic syndrome patients following a 12 hour fast;

Figure 8 is a graph of the average lipid body area per monocyte cell of leukocyte samples obtained from two healthy human subjects and two metabolic syndrome patients following a 12 hour fast;

Figure 9 is a graph of the average number of lipid bodies per monocyte cell of leukocyte samples obtained from two healthy human subjects and two diabetic patients following a 12 hour fast and one, 1.5, two and 2.5 hours thereafter, following consumption of a high-glucose meal;

Figure 10 is a graph of the average lipid body area per monocyte cell of leukocyte samples obtained from two healthy human subjects and two diabetic patients following a 12 hour fast and one, 1.5, two and 2.5 hours thereafter, following consumption of a high-glucose meal;

Figure 11 is a graph of the average number of lipid bodies per neutrophil cell of leukocyte samples obtained from three control mice and three atherosclerotic ApoE knockout mice;

Figure 12 is a graph of the average lipid body area per neutrophil cell of leukocyte samples obtained from three control mice and three atherosclerotic ApoE knockout mice; and

Figure 13 is a graph of the statistical distribution of the average number of lipid bodies per neutrophil cell vs. the average lipid body area per neutrophil cell of leukocyte samples obtained from three control mice and three atherosclerotic ApoE knockout mice.

DETAILED DESCRIPTION

In the following description, various aspects of the present invention will be described. For purposes of explanation, specific configurations and details are set forth in order to provide a thorough understanding of the present invention. However, it will also be apparent to one skilled in the art that the present invention may be practiced

without the specific details presented herein. Furthermore, well known features may be omitted or simplified in order not to obscure the present invention.

Embodiments of the invention may provide a non-invasive method for diagnosing and detecting a metabolic malady or abnormality by examination of a lipid body in a leukocyte sample, such as in a monocyte population, a neutrophil cell population, an eosinophil cell population, a basophil cell population, a lymphocyte population or a macrophage population of a human or an animal subject.

In one embodiment of the invention a metabolic malady or abnormality is, for example, without limitation, a metabolic disease, a metabolic disorder, atherosclerosis, glucose intolerance, type II diabetes, metabolic syndrome, insulin resistance, pre-diabetes, lipotoxicity, fatty liver, steatohepatitis, steatosis, obesity, a stroke, a cardio-vascular disease, hyperlipidemia, or a metabolic malady complication, such as a diabetes complication, such as diabetic retinopathy and diabetic nephropathy or any other factor pertaining to a metabolic disease or disorder. Other abnormalities or maladies may be detected and/or diagnosed.

Metabolic malady is among the most common chronic human diseases. The etiology of this malady is considered to be multifactorial, involving genetic and environmental effects.

Accumulation of lipid droplets, which is identical to the term lipid bodies, such as triglyceride accumulation, in various body tissues, such as muscle, liver or pancreas tissue, is considered to be an important factor of organ specific insulin resistance leading to metabolic malady development. Emerging evidence indicates a role for inflammation as a pathogenetic event in a metabolic malady.

Thus, taking into consideration lipid body accumulation in inflamed leukocytes and the role of inflammation in the development of a metabolic malady the assumption is made that quantification and morphological characterization of lipid accumulation, such as triglyceride or eicosanoid accumulation, in peripheral leukocytes and macrophages, such as monocytes, may provide a non-invasive method for analyzing a metabolic malady by examination and/or metrology of a lipid body or bodies in a leukocyte sample.

Reference is now made to Figure 1A, which is a simplified flowchart of an embodiment of a method for detecting a metabolic malady or abnormality. As seen in

Figure 1A, in block 10 a blood sample with a leukocyte may be inserted into an examination system. Analysis of a quantitative feature of a lipid body in the leukocyte may be performed, as shown in block 12. As seen in block 14, if a value of the quantitative feature is significantly different than a threshold a metabolic malady may be detected, as seen in block 16. If a value of the quantitative feature is not significantly different than a threshold a metabolic malady may not detected, as seen in block 18.

It is appreciated that the embodiment of the method described in Figure 1A may be performed in any suitable manner and in any suitable order. Other operations may be used.

A method according to one embodiment of the invention for detecting and/or diagnosing a metabolic malady or abnormality in a subject may include the steps of introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from the subject and analyzing a quantitative feature of a lipid body in the leukocyte, wherein if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a threshold, the subject is diagnosed and/or detected as having a metabolic malady.

A method according to one embodiment of the invention includes introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from a subject and analyzing a quantitative feature of a lipid body in the leukocyte.

A method for metrology of a quantitative feature of a lipid body in a leukocyte according to one embodiment of the invention includes introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from a subject and analyzing the quantitative feature of the lipid body in the leukocyte.

A method according to one embodiment of the invention includes obtaining a whole blood sample from a human or animal subject by any suitable method, typically by venipuncture. The whole blood is separated to obtain leukocytes, by any suitable method, such as by incubating the whole blood sample thus causing a leukocyte portion to generally separate from an erythrocyte portion. The removed resulting leukocyte portion may be centrifuged thereafter. It is appreciated that the leukocytes may be obtained from any suitable body compartment, such as the urine.

The leukocytes may be prepared for analysis using any conventional fixation method, such as addition of aldehydes, such as formaldehyde, glutaraldehyde, addition of methanol, addition of osmium tetroxide or a combination thereof, prior to imaging. Alternatively, the leukocyte samples may be analyzed and imaged without prior fixation.

Analysis may be performed by any suitable method such as by examination of the sample in an examination system, such as a metrology system. A metrology system is a system for measuring a sample, such as, without limitation, electron microscopy, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), light microscopy, such as confocal light microscopy, fluorescence, typically using Nile-Red staining, fluorescence-activated cell sorter (FACS) or enzyme-linked immunosorbent assay (ELISA).

Alternatively, analysis may include imaging the samples in an examination system, such as an imaging system, and thereafter measuring the sample employing any suitable method, such as by measuring the sample during human visualization.

Analysis of a lipid body includes in one embodiment of the invention measuring a quantitative feature of the lipid body in the leukocyte sample.

Resulting value or values of a measurement of the quantitative features may be used as data for diagnosing, detecting or predicting a metabolic malady presence or severity in the subject. If a value of a quantitative feature is significantly different in comparison with a threshold, the subject is diagnosed and/or detected as having, or being susceptible to developing, the metabolic malady.

It is appreciated that the term "quantitative feature" may represent any measurable feature of the lipid body, such as, without limitation, the average, median or total lipid body content, area, volume, weight or number of lipid bodies in a cell or in a plurality of cells, the maximal or minimal lipid body content, area, weight or volume per cell or cells and/or the maximal or minimal number of lipid bodies per cell or cells, or any lipid body size, such as the diameter or radius, any morphological feature or a combination thereof.

The lipid body content is the total lipid body area in a single lipid body or a plurality of lipid bodies.

In one embodiment of the invention the measured lipid body area is a surface confined within a great circle of a sphere-like shape defining the lipid body. A great circle is conventionally defined as a section of a sphere that contains a diameter of the sphere. Additionally, the measured lipid body area may be any suitable measurement, such as a cross sectional area of the lipid body.

The terms "significantly different" or “significant difference” may represent any measurable difference between a quantitative feature of a lipid body of a blood sample derived from a subject and a quantitative feature of a lipid body of a blood sample derived from a control. In one embodiment, this difference may be approximately 30% or less. Alternatively, the difference may represent an approximately 30%, 40%, 50%, 70%, 100% or more, difference. Additionally the difference may be, without limitation, an increase of a factor of approximately 1.5, 2, 3, 4, 5, or 6 or more of the quantitative feature of a lipid body of a blood sample derived from the subject compared to a quantitative feature of a lipid body of a blood sample derived from a control.

The significant deference may be a substantial difference or a considerable difference.

For example, if the lipid body area in the leukocyte sample from the human or animal subject is significantly higher than the lipid body area in a leukocyte sample derived from a control, the subject is diagnosed and/or detected as having a metabolic malady.

It is noted that the term "control" refers in the application to a healthy subject, or to data derived and calculated from one or more healthy subjects. The subject may be an animal or a human.

Analyzing also includes in any embodiment of the invention calculating the quantitative feature of the lipid body in the blood sample derived from the subject and comparing the resulting value of the quantitative feature of the blood sample from the subject with a value of a quantitative feature of the blood sample from a control.

The threshold may be a constant, wherein if, for example, the lipid body area of the subject is higher than the threshold constant, the subject is diagnosed and/or detected as having a metabolic malady. It is appreciated that the threshold may vary in different populations and/or in accordance with different environmental circumstances.

Additionally, for example, a statistical distribution of the number of lipid bodies per lipid body area differing from the statistical distribution of a control subject may indicate that the subject has a metabolic malady. Any other indicative statistical distribution may be used to diagnose and/or detect a metabolic malady in the subject.

5 It is further appreciated that the data may result from analysis of a quantitative feature of a leukocyte-containing sample derived from a subject and comparison with a leukocyte-containing sample derived from the subject following an administrated procedure, wherein the quantitative feature of a lipid body in a leukocyte sample from the subject following an administrated procedure may be significantly higher or 10 significantly lower, according to the administrated procedure, in comparison with the quantitative feature of the lipid body in a leukocyte sample from the subject prior to administration of the procedure.

Typically, the term "administrated procedure" may be a fast, physical activity, nutrient supplementation, medical treatment or progression of time, such as a time 15 difference from days to weeks or months or a combination thereof.

Medical treatment may include, without limitation, a treatment for a metabolic malady. It is appreciated that any suitable administrated procedure may be employed.

It is appreciated that the various administrated procedures may cause different reactions in a quantitative feature of a lipid body. For example, a quantitative feature of 20 a lipid body in a leukocyte sample from a human or animal subject following a medical treatment, for example, may be significantly lower than a quantitative feature of a lipid body in a leukocyte sample derived from the subject prior to the medical treatment.

Alternatively, for example, if an increase of a value of a quantitative feature of a lipid body of a subject, which increase is caused by administration of a procedure, is 25 significantly higher than an increase in a value of a quantitative feature of a lipid body of a control, which increase is caused by administration of the procedure, the subject is diagnosed and/or detected as having a metabolic malady.

It is appreciated that a method according to one embodiment may be used for stratifying subjects in accordance with the subject's compatibly with a suitable 30 administrated procedure or intervention.

A quantitative feature of lipid bodies of leukocyte samples derived from a group of subjects following a procedure administrated thereto, such as, for example,

administration of a pharmaceutical agent or a diet, may be analyzed so as to evaluate the compatibility and/or effectivity of the administered procedure in metabolic malady prevention and/or treatment for the group of subjects. For example, if a number of lipid droplets of blood samples derived from a group of human subjects treated by a pharmaceutical agent or a diet, for example, is smaller than a number of lipid droplets of blood samples derived from the group of human subjects prior to pharmaceutical treatment or diet, the human subject group may be stratifies as being compatible and/or treated by the pharmaceutical agent or diet.

Additionally, analysis of a quantitative feature of lipid bodies of leukocyte samples derived from a subject or subjects may be used to stratify the subject or subjects to determine a course of treatment suitable for the subject or subjects. For example, if a number of lipid droplets of blood samples derived from a human subject or subjects is significantly different than a threshold, the subject or subjects may be stratified as a subject or subjects that may pursue a specific course of treatment, such as treatment by a pharmaceutical agent or a specific diet.

Analyzing also includes in any embodiment of the invention calculating the quantitative feature of the lipid body in the blood sample derived from the subject and comparing the resulting value of the quantitative feature of the lipid body in the blood sample derived from the subject with a value of a quantitative feature of the lipid body in the blood sample from the subject following an administrated procedure.

It is appreciated that analysis may be performed on any lipid containing component of the leukocyte.

A method according to one embodiment may be used for early detection and/or diagnosis of the metabolic malady or abnormality in a subject. The subject may be a human subject being at risk for developing a metabolic malady, such as, without limitation, being obese or having a family history of a metabolic malady or any other risk for developing a metabolic malady. In one embodiment of the invention, the subject may be an animal, such as an animal genetically engineered to have a tendency to develop a metabolic malady or an animal induced to be prone to a metabolic malady, such as for example without being limited, by being fed a high-fat diet.

Reference is now made to Figure 1B, which is a simplified flowchart of an embodiment of a method for early detection of a metabolic malady or abnormality. As

seen in Figure 1B, in block 20 a blood sample with a leukocyte may be inserted into an examination system. Analysis of a quantitative feature of a lipid body in the leukocyte may be performed, as shown in block 22. As seen in block 24, if a value of the quantitative feature is significantly different than a threshold a susceptibility to develop 5 a metabolic malady may be detected, as seen in block 26. If a value of the quantitative feature is not significantly different than a threshold a susceptibility to develop a metabolic malady may not be detected, as seen in block 28.

It is appreciated that the embodiment of the method described in Figure 1B may be performed in any suitable manner and in any suitable order. Other operations may be 10 used.

A method according to one embodiment of the invention for early detection and/or diagnosis of a metabolic malady or abnormality may include the steps of introducing a blood sample containing a leukocyte into an examination system, the blood sample being derived from a subject and analyzing a quantitative feature of a lipid 15 body in the leukocyte, wherein if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a threshold, the subject is detected as having a susceptibility for developing a metabolic malady.

Early detection and/or diagnosis may be performed, for example, by measuring 20 any quantitative feature of the lipid bodies in the leukocyte sample.

Resulting value or values of the measurements may be used as data for early detection and/or diagnosis of a metabolic malady.

For example, if the lipid body content in the leukocyte sample from the human or animal subject is higher than the lipid body content in a leukocyte sample derived 25 from a control, the subject is detected as having a susceptibility for developing a metabolic malady.

It is appreciated that any suitable value of significant difference or threshold, such as the differences and thresholds described hereinabove, may be employed. Additionally, any suitable representation of the quantitative feature may be employed, 30 as described hereinabove.

It is further appreciated that the data may result from comparing a value of a quantitative feature of a leukocyte-containing sample derived from a subject with a

value of a quantitative feature of a leukocyte-containing sample derived from the subject following an administrated procedure, as described hereinabove.

Reference is now made to Figure 1C, which is a simplified flowchart of an embodiment of a method for assessing the efficiency and/or efficacy of a ligand for
5 metabolic malady or abnormality prevention or treatment. As seen in Figure 1C, in block 30 a ligand is administrated to a subject. In block 32 a blood sample with a leukocyte is obtained from the subject. In block 34 the blood sample may be inserted into an examination system. Analysis of a quantitative feature of a lipid body in the leukocyte may be performed, as shown in block 36. As seen in block 38, if a value of
10 the quantitative feature is lower than a value of a quantitative feature derived from an untreated subject the ligand may be efficacious and/or efficient for metabolic malady treatment, as seen in block 40. If a value of the quantitative feature is not significantly lower than a value of a quantitative feature derived from an untreated subject the ligand may be inefficacious or inefficient for metabolic malady treatment, as seen in block 42.
15 It is appreciated that the embodiment of the method described in Figure 1C may be performed in any suitable manner and in any suitable order. Other operations may be used.

A method according to one embodiment may be used for assessing the efficiency and/or efficacy of a ligand for metabolic malady or abnormality prevention or
20 treatment including the steps of obtaining a blood sample containing a leukocyte from a subject with a metabolic malady following administration of the ligand to the subject, introducing the blood sample into an examination system and analyzing a quantitative feature of a lipid body in the leukocyte of the blood sample, wherein if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample is lower than
25 a value of a quantitative feature of a lipid body in a leukocyte of a blood sample of an untreated subject with a metabolic malady, the ligand is detected as efficacious for treatment or prevention of a metabolic malady.

A ligand may be, for example, a chemical reagent, a nucleic acid, a drug, a nucleic acid, ribozymes, RNA, an antibody, a peptide or a compound. The ligand may
30 be for example, a potential drug candidate or a "lead" compound or molecule for treating a metabolic malady.

Assessment of the ligand may be performed, for example, by measuring any quantitative feature of the lipid bodies in the leukocyte sample. The leukocyte sample may be a sample derived from a human subject or an animal.

Resulting value or values of the measurements may be used as data for assessing 5 the efficiency and/or efficacy of a ligand for metabolic malady prevention or treatment.

For example, if the lipid body area in the leukocyte sample from an untreated subject, with a metabolic malady or animal model for the same, is higher than the lipid body area in a leukocyte sample derived from a subject with a metabolic malady and which the ligand is administrated thereto, the ligand is detected as being efficient and/or 10 efficacious for treatment or prevention of a metabolic malady.

Alternatively, if the value of the lipid body area in the leukocyte sample from an animal subject with the ligand administrated thereto and prior to being induced with a metabolic malady, is substantially equal to the value of the lipid body area in a leukocyte sample derived from the animal subject following induction of a metabolic 15 malady thereto, the ligand is detected as efficient and/or efficacious for treatment or prevention of a metabolic malady. It is further appreciated that the data may result from comparing a quantitative feature of a leukocyte-containing sample derived from a subject with a value of a quantitative feature of a leukocyte-containing sample derived from the subject following administrating the ligand to the subject.

20 It is appreciated that any suitable value of significant difference or threshold, such as the differences and thresholds described hereinabove, may be employed. Additionally, any suitable representation of the quantitative feature may be employed, as described hereinabove.

A method according to one embodiment may be used for monitoring the 25 metabolic malady. The subject may be a human or animal subject having a metabolic malady.

Reference is now made to Figure 1D, which is a simplified flowchart of an embodiment of a method for monitoring a metabolic malady or abnormality in a subject. As seen in Figure 1D, in block 50 a blood sample with a leukocyte derived 30 from a subject may be inserted into an examination system. Analysis of a quantitative feature of a lipid body in the leukocyte may be performed, as shown in block 52. As seen in block 54, if a value of the quantitative feature is significantly higher than a

threshold the metabolic malady in the subject may have deteriorated, as seen in block 56. If a value of the quantitative feature is not significantly higher than a threshold the metabolic malady in the subject may have improved or may be unchanged, as seen in block 58.

5 It is appreciated that the embodiment of the method described in Figure 1D may be performed in any suitable manner and in any suitable order. Other operations may be used.

A method according to one embodiment for monitoring a metabolic malady or abnormality includes introducing a blood sample containing a leukocyte into an 10 examination system, the blood sample being derived from a subject and analyzing a quantitative feature of a lipid body in the leukocyte by evaluating if a value of the quantitative feature of the lipid body in the leukocyte of the blood sample derived from the subject is significantly different than a threshold, thereby monitoring a metabolic malady.

15 Monitoring may be performed by, for example, measuring any quantitative feature of the lipid body in the leukocyte sample.

Resulting value or values of the measurements of the quantitative feature may be used, for example, as data for monitoring the metabolic malady. The value of the quantitative feature may be compared to a predetermined threshold for monitoring the 20 metabolic malady.

For example, if the value of the lipid body area in the leukocyte sample derived from the subject, prior to a procedure administrated to the subject, is significantly higher, than the value of the lipid body area in a leukocyte sample derived from the subject, following the procedure administrated to the subject, an improvement in the 25 subject with the metabolic malady is monitored.

The administrated procedure may be any suitable procedure, such as described hereinabove and including progression of time, such as passing of a few days, weeks or months. Thus, if the value of the lipid body area in the leukocyte sample derived from the subject, prior to progression of time, is significantly different, such as higher, than a 30 value of the lipid body area in a leukocyte sample derived from the subject, following the progression of time, an improvement of the subject with the metabolic malady is detected.

It is appreciated that any suitable value of significant difference or threshold, such as the significant differences and thresholds described hereinabove, may be employed. Additionally, any suitable representation of the quantitative feature may be employed, as described hereinabove.

5 It is noted that data obtained employing other conventional methods may be used jointly with data including the values of the quantitative features, in the embodiments described hereinabove, for metabolic malady analysis. For example, a number of lipid bodies and a level of fasting glucose may be used together for early detection and/or diagnosis of a metabolic malady.

10 In the examples described hereinbelow lipid bodies were imaged in a scanning electron microscope (SEM) using a sample container and attaching a leukocyte sample to a membrane of the sample container by use of a pipette.

The sample container may be a sample container, such as a SEM compatible sample container, for example, disclosed in embodiments described in PCT patent 15 application PCT/IL2003/001054 and published as WO/2004/075209, which is hereby incorporated by reference herein in its entirety; PCT patent application PCT/IL03/00457 and published as WO03/104848, which is hereby incorporated by reference herein in its entirety and PCT patent application PCT/IL03/00454 and published as WO03/104846, which is hereby incorporated by reference herein in its entirety. The membrane may be 20 a membrane, such as a membrane of a SEM compatible sample container, for example, disclosed in embodiments described in PCT patent application PCT/IL2003/001054 and published as WO/2004/075209, which is hereby incorporated by reference herein in its entirety; PCT patent application PCT/IL03/00457 and published as WO03/104848, which is hereby incorporated by reference herein in its entirety and PCT patent 25 application PCT/IL03/00454 and published as WO03/104846, which is hereby incorporated by reference herein in its entirety. The pipette may be a pipette, such as a pipette, for example, disclosed in embodiments described in PCT patent application PCT/IL2003/001054 and published as WO/2004/075209, which is hereby incorporated by reference herein in its entirety; PCT patent application PCT/IL03/00457 and 30 published as WO03/104848, which is hereby incorporated by reference herein in its entirety and PCT patent application PCT/IL03/00454 and published as WO03/104846,

which is hereby incorporated by reference herein in its entirety. Other suitable membranes, containers and pipettes may be used.

Examination of the leukocyte sample in the sample container in the SEM may reveal differences in material composition between different regions of the samples. For example, efficiency of electron backscattering depends on the atomic number (Z) of the constituent atoms. Thus, lipid-rich regions of the samples, composed mostly of carbon, may be distinguished from aqueous regions, composed mostly of oxygen. In another embodiment, substances including heavy atoms may be used to stain the samples using conventional methods, providing additional contrast between constituents of the samples.

Thus an embodiment of the method may provide an analytical tool for a metabolic malady, which may be employed for a plurality of purposes, such as, without limitation, early detection and/or diagnosis of a metabolic malady or abnormality; a diagnostic biomarker for a metabolic malady; therapy monitoring, such as drug therapy or administration of a weight loss diet in preclinical studies in clinical studies and in patients; monitoring the progression of a metabolic malady; monitoring the deterioration of a subject with a metabolic malady; aid in drug development by providing an analytical tool for target identification, target validation, screening of agents, such as inhibitors and modulators, hit to lead optimization and animal studies and patient stratification and classification.

The analytical tool may be provided, for example, in a form of a kit for metabolic malady analysis of a human or animal subject including a sample container for inserting the leukocyte-containing blood sample therein and data for analysis of a quantitative feature of the lipid body so as to analyze the metabolic malady of the subject. The data may be a threshold of the quantitative feature of the lipid body, the metabolic malady analysis is performed by comparing the threshold with a value of the quantitative feature of the lipid body.

The quantitative feature may be, for example, a lipid body area, a total lipid body area, an average lipid body area, a median lipid body area, a maximal lipid body area in at least one leukocyte, a minimal lipid body area in at least one leukocyte, a lipid body content, an average lipid body content, a median lipid body content, a maximal lipid body content in at least one leukocyte, a minimal lipid body content in at least one

leukocyte, a number of lipid bodies, a total number of lipid bodies, an average number of lipid bodies, a median number of lipid bodies, a maximal number of lipid bodies in at least one leukocyte, or a minimal number of lipid bodies in at least one leukocyte, a lipid body weight, a lipid body volume a lipid body size, a lipid body diameter, a lipid 5 body radius or a combination thereof, as described hereinabove.

Additionally, a statistical distribution of the number of lipid bodies per lipid body area differing from the statistical distribution of a control subject may be employed for metabolic malady analysis. Furthermore, a statistical distribution of the number of lipid bodies per lipid body area differing from the statistical distribution of 10 the subject, following an administrated procedure, as described hereinabove, may be employed for metabolic malady analysis. Any other indicative statistical distribution may be used to analyze a metabolic malady in the subject.

Analysis may be performed by any suitable manner such as by imaging in a metrology system, as described hereinabove. Image analysis functionality, such as 15 image analysis software, may be provided.

The kit may be used for any suitable purpose, such as for metabolic malady detection and/or diagnosis, early detection and/or diagnosis of a metabolic malady, assessing the efficiency of a ligand for metabolic malady prevention or treatment, or monitoring the deterioration of the subject with the metabolic malady, as described 20 hereinabove, for example.

Reference is now made to Figure 2A, which is a simplified pictorial illustration of a kit 100 constructed and operative in accordance with one embodiment. As seen in Figure 2A, the kit 100 includes a sample container 104 for inserting a blood sample containing a leukocyte therein. A paper 110 may include instructions with data 120 for 25 analysis of a quantitative feature of a lipid body so as to detect the presence of a metabolic malady.

Reference is now made to Figure 2B, which is a simplified pictorial illustration of a kit 200 constructed and operative in accordance with one embodiment. As seen in Figure 2B, the kit 200 includes a sample container 204, for inserting a blood sample 30 containing a leukocyte therein. Sample container 204 may be identical to sample container 104 in Figure 2A. Instructions may be displayed on an electronic sheet 210 on a display 212 of a computer 214 or a memory device, such as a hard disk or electronic

memory (e.g., RAM, ROM, etc), for example. The electronic sheet 210 may display data 220 for analysis of a quantitative feature of a lipid body so as to detect the presence of a metabolic malady.

It is appreciated that data may be presented or provided in any suitable manner, such as by transmission of the data via a telephone, for example. Data 120 and 220 of respective Figures 2A and 2B may include any suitable data for analysis of a quantitative feature of a lipid body, such as, for example, values of a quantitative feature of a lipid body, thresholds, graphs and statistical analysis, such as described hereinabove, for example.

The data may be provided in any suitable form, as a list of values of quantitative features, for example.

Kits 100 and 200 of respective Figures 2A and 2B may include any suitable sample container, as described hereinabove.

As can be seen in Figure 3 (A and B), which is a micrograph of a neutrophil-containing sample of a representative healthy human subject (A) and a representative metabolic syndrome patient (B), the lipid body content in the sample taken from the metabolic syndrome patient is significantly larger than in the sample taken from the healthy subject. As can be seen in Figure 3B (the metabolic syndrome patient), there is a significantly larger number of lipid bodies, distinguished from the cell surroundings as bright spots, approximately 13 bodies, in comparison with Figure 3A (the healthy human subject) wherein approximately two lipid bodies (the bright spots) can be seen. Furthermore, it can be seen that the average size of the lipid body area in Figure 1B (the metabolic syndrome patient) is significantly larger, approximately 1.5 μm^2, in comparison with Figure 3A (the healthy human subject) wherein the average lipid body area is approximately 0.5 μm^2.

Similarly, it can be seen in Figure 4 (A and B), which is a micrograph of a monocyte-containing sample of a healthy human subject (A) and a metabolic syndrome patient (B), the lipid body content in the sample taken from the metabolic syndrome patient is significantly larger than in the sample taken from the healthy subject. As can be seen in Figure 4B (the metabolic syndrome patient), there is a significantly larger number of lipid bodies (bright spots), approximately eight, in comparison with Figure 4A (the healthy human subject), wherein no lipid bodies are seen.

EXAMPLE 1

Whole blood samples were obtained from a group of two healthy human subjects and a group of two metabolic syndrome patients. Whole blood samples were taken from each human subject following a 12 hour fast.

Experimental Procedure:

A whole blood sample was collected in tubes including a solution of sodium citrate. Four hours after drawing the whole blood sample from each human subject, approximately one milliliter of whole blood was mixed with a dextran solution of 335 microliters and incubated at a temperature of approximately 37°C for approximately 30 minutes. The dextran solution included 6% dextran, 3% glucose and 0.9% NaCl and was stored at a temperature of approximately -20°C prior to mixture with the whole blood sample.

Following incubation, the whole blood sample separated into an erythrocyte portion and a leukocyte portion. Approximately 335 microliters of the leukocyte portion was removed from the erythrocyte portion and approximately one milliliters of phosphate buffer solution (PBS), including 0.89 mM calcium and 0.49 mM magnesium was added thereto. The resulting leukocyte sample was centrifuged at a speed of approximately 1500 rpm for approximately ten minutes. Thereafter, resulting supernatant was removed from the leukocyte sample. Approximately 150 microliters of PBS including 0.89 mM calcium and 0.49 mM magnesium was added to the leukocyte sample. Approximately 50 microliters are removed from a resulting solution and diluted with approximately 750 microliters of PBS including 0.89 mM calcium and 0.49 mM magnesium.

A portion of a resulting solution, including 15 microliters of the leukocyte sample, was attached to a membrane of a sample container by use of a pipette and was subsequently incubated for approximately 15 minutes at room temperature.

Thereafter, the leukocyte sample was fixed with a solution of 2% paraformaldehyde and 0.1% glutaraldehyde diluted in a PBS solution for approximately

30 minutes at room temperature. Subsequently, the leukocyte sample was washed four times with a PBS solution and four times with double distilled water.

Prior to imaging of the leukocyte sample within the sample container the leukocyte sample was stained with a solution including 0.5% OsO_4 for approximately
5 30 minutes at room temperature. Subsequently, the leukocyte sample was washed four times with double distilled water. The leukocyte sample was then stained with a solution including 0.5% uranyl acetate for approximately five minutes at room temperature. Subsequently, the leukocyte sample was washed four times with double distilled water.

10

Experimental Results:

As can be seen in Figure 5, the average number of lipid bodies per neutrophil cell of a leukocyte sample is approximately 5.66 with a standard deviation of 0.457 in
15 the healthy human subject group. The average number of lipid bodies per neutrophil cell of a leukocyte sample is approximately 8.88 with a standard deviation of 0.96 in the metabolic syndrome patient group. It can be seen that the average number of lipid bodies per neutrophil cell is significantly higher in the metabolic syndrome patient group than in the healthy human subject group. Correspondently, the average area of
20 lipid bodies per neutrophil cell of a leukocyte sample is approximately 0.74 μm^2 with a standard deviation of 0.84 μm^2 in the healthy human subject group and approximately 1.973 μm^2 with a standard deviation of 0.016 μm^2 in the metabolic syndrome patient group. It can be seen that the average lipid body area per neutrophil cell is significantly higher in the metabolic syndrome patient group than in the healthy human subject group
25 (Figure 6).

As can be seen in Figure 7, the average number of lipid bodies per monocyte cell of a leukocyte sample is approximately 2.09 with a standard deviation of 0.41 in the healthy human subject group. The average number of lipid bodies per monocyte cell of a leukocyte sample is approximately 4.56 with a standard deviation of 0.37 in the
30 metabolic syndrome patient group. It can be seen that the average number of lipid bodies per monocyte cell is significantly higher in the metabolic syndrome patient group than in the healthy human subject group. Correspondently, the average area of

lipid bodies per monocyte cell of a leukocyte sample is approximately 0.161 μm^2 with a standard deviation of 0.064 μm^2 in the healthy human subject group and approximately 0.444 μm^2 with a standard deviation of 0.071 μm^2 in the metabolic syndrome patient group. It can be seen that the average lipid body area per monocyte cell is significantly higher in the metabolic syndrome patient group than in the healthy human subject group (Figure 8).

EXAMPLE 2

Whole blood samples were obtained from a group of two healthy human subjects and a group of two type II diabetes patients, following a 12 hour fast, one, 1.5, two and 2.5 hours thereafter, following consumption of a high-glucose meal including 75 grams of dextrose.

Experimental Procedure:

A whole blood sample was collected in tubes including a solution of sodium citrate. Four hours after drawing the whole blood sample from each human subject, approximately one milliliter of whole blood was mixed with a dextran solution of 335 microliters and incubated at a temperature of approximately 37°C for approximately 30 minutes. The dextran solution included 6% dextran, 3% glucose and 0.9% NaCl and was stored at a temperature of approximately -20°C prior to mixture with the whole blood sample.

Following incubation, the whole blood sample separated into an erythrocyte portion and a leukocyte portion. Approximately 335 microliters of the leukocyte portion was removed from the erythrocyte portion and approximately one milliliters of PBS, including 0.89 mM calcium and 0.49 mM magnesium was added thereto. The resulting leukocyte sample was centrifuged at a speed of approximately 1500 rpm for approximately ten minutes. Thereafter, resulting supernatant was removed from the leukocyte sample. Approximately 150 microliters of PBS including 0.89 mM calcium and 0.49 mM magnesium was added to the leukocyte sample. Approximately 50

microliters are removed from a resulting solution and diluted with approximately 750 microliters of PBS including 0.89 mM calcium and 0.49 mM magnesium.

A portion of a resulting solution, including 15 microliters of the leukocyte sample, was attached to a membrane of a sample container by use of a pipette and was 5 subsequently incubated for approximately 15 minutes at room temperature.

Thereafter, the leukocyte sample was fixed with a solution of 2% paraformaldehyde and 0.1% glutaraldehyde diluted in a PBS solution for approximately 30 minutes at room temperature. Subsequently, the leukocyte sample was washed four times with a PBS solution and four times with double distilled water.

10 Prior to imaging of the leukocyte sample within the sample container the leukocyte sample was stained with a solution including 0.5% OsO_4 for approximately 30 minutes at room temperature. Subsequently, the leukocyte sample was washed four times with double distilled water. The leukocyte sample was then stained with a solution including 0.5% uranyl acetate for approximately five minutes at room 15 temperature. Subsequently, the leukocyte sample was washed four times with double distilled water.

Experimental Results:

20 As can be seen in Figure 9, the average number of lipid bodies per monocyte of a leukocyte sample is approximately 2.6202 with a standard deviation of 0.0165 in the healthy human subject group, following a 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 5.44 with a standard deviation of 0.1507 in the diabetic patient group, following a 12 hour fast. The average 25 number of lipid bodies per monocyte of a leukocyte sample is approximately 2.04 with a standard deviation of 0.1698 in the healthy human subject group, one hour after consumption of the high-glucose meal following the 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 4.53 with a standard deviation of 0.1341 in the diabetic patient group, one hour after consumption 30 of the high-glucose meal following the 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 1.54 with a standard deviation of 0.1084 in the healthy human subject group, 1.5 hours after consumption of the high-

glucose meal following the 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 2.36 with a standard deviation of 0.6414 in the diabetic patient group, 1.5 hours after consumption of the high-glucose meal following the 12 hour fast.

5 The average number of lipid bodies per monocyte of a leukocyte sample is approximately 1.63 with a standard deviation of 0.1149 in the healthy human subject group, two hours after consumption of the high-glucose meal following the 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 3.39 with a standard deviation of 0.389 in the diabetic patient group, two 10 hours after consumption of the high-glucose meal following the 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 1.17 with a standard deviation of 0.1788 in the healthy human subject group, 2.5 hours after consumption of the high-glucose meal following the 12 hour fast. The average number of lipid bodies per monocyte of a leukocyte sample is approximately 3.81 with 15 a standard deviation of 0.14 in the diabetic patient group, 2.5 hours after consumption of the high-glucose meal following the 12 hour fast. It can be seen that the average number of lipid bodies per monocyte is significantly higher in the diabetic patient group than in the healthy human subject group. A fluctuation in the number of lipid bodies is seen at the various times following consumption of the high-glucose meal, nevertheless the 20 range of values of the number of lipid bodies in the diabetic group it significantly higher than in the healthy subject group.

Correspondently, the average lipid body area per monocyte of a leukocyte sample is approximately 0.23 μm^2 with a standard deviation of 0.04 μm^2 in the healthy human subject group, following a 12 hour fast. The average lipid body area per 25 monocyte of a leukocyte sample is approximately 0.52 μm^2 with a standard deviation of 0.0227 μm^2 in the diabetic patient group, following a 12 hour fast. The average lipid body area per monocyte of a leukocyte sample is approximately 0.2 μm^2 with a standard deviation of 0.0615 μm^2 in the healthy human subject group, one hour after consumption of the high-glucose meal following the 12 hour fast. The average lipid 30 body area per monocyte of the leukocyte sample is approximately 0.48 μm^2 with a standard deviation of 0.0121 μm^2 in the diabetic patient group, one hour after consumption of the high-glucose meal following the 12 hour fast. The average lipid

body area per monocyte of a leukocyte sample is approximately 0.13 μm^2 with a standard deviation of 0.0204 μm^2 in the healthy human subject group, 1.5 hours after consumption of a high-glucose meal following the 12 hour fast. The average lipid body area per monocyte of a leukocyte sample is approximately 0.2 μm^2 with a standard deviation of 0.0805 μm^2 in the diabetic patient group, 1.5 hours after consumption of the high-glucose meal following the 12 hour fast.

The average lipid body area per monocyte of a leukocyte sample is approximately 0.13 μm^2 with a standard deviation of 0.0228 μm^2 in the healthy human subject group, two hours after consumption of the high-glucose meal following the 12 hour fast. The average lipid body area per monocyte of a leukocyte sample is approximately 0.33 μm^2 with a standard deviation of 0.118 μm^2 in the diabetic patient group, two hours after consumption of the high-glucose meal following the 12 hour fast. The average lipid body area per monocyte of a leukocyte sample is approximately 0.11 μm^2 with a standard deviation of 0.0114 μm^2 in the healthy human subject group, 2.5 hours after consumption of the high-glucose meal following the 12 hour fast. The average lipid body area per monocyte of a leukocyte sample is approximately 0.37 μm^2 with a standard deviation of 0.0697 μm^2 in the diabetic patient group, 2.5 hours after consumption of the high-glucose meal following the 12 hour fast. It can be seen that the average lipid body area per monocyte is significantly higher in the diabetic patient group than in the healthy human subject group. A fluctuation in the lipid body area is seen at the various times following consumption of the high-glucose meal, nevertheless the range of values of the lipid body area in the diabetic group it significantly higher than in the healthy subject group (Figure 10).

The values of the number of lipid bodies and lipid body area correspond to results of a fasting glucose test of the two healthy human subjects and two diabetic patients. The respective values of the fasting glucose of the two healthy human subjects and two diabetic patients are 90 mg/dL, 91 mg/dL, 147 mg/dL and 182 mg/dL, wherein the threshold for diagnosing a human subject as diabetic is approximately 100 mg/dL.

EXAMPLE 3

Whole blood samples were obtained from a group of three control mice and a group of three atherosclerotic ApoE knockout mice.

Experimental Procedure:

5

A whole blood sample was collected in tubes including a solution of ethylene diamine tetraacetic acid (EDTA). One hour after drawing the whole blood, approximately 300 microliters of whole blood were mixed with 900 microliters of an erythrocyte-lysis solution and incubated at room temperature for approximately 5
10 minutes, with occasional mixing.

The erythrocyte-lysis solution is commercially available under the brand name PUREGENE from Gentra Systems, Inc. at 13355 10th Avenue N, Suite 120, Minneapolis, MN 55441,USA.

Following incubation, the whole blood sample was centrifuged at a speed of
15 approximately 1200 rpm for approximately five minutes. Thereafter, resulting supernatant was removed from the sample, thus a portion containing leukocytes remains and forms a leukocyte sample. Approximately 100 microliters of PBS including 0.89 mM calcium and 0.49 mM magnesium was added to the leukocyte sample.

A portion including 15 microliters of a leukocyte sample was attached to a
20 membrane of a sample container by use of a pipette and was subsequently incubated for approximately 60 minutes at room temperature.

Thereafter, the leukocyte sample was fixed with a solution of 2% paraformaldehyde and 0.1% glutaraldehyde diluted in a PBS solution for approximately 30 minutes at room temperature. Subsequently, the leukocyte sample was washed four
25 times with a PBS solution and four times with double distilled water.

Prior to imaging of the leukocyte sample within the sample container the leukocyte sample was stained with a solution including 0.5% OsO_4 for approximately 30 minutes at room temperature. Subsequently, the leukocyte sample was washed four times with double distilled water. The leukocyte sample was then stained with a
30 solution including 0.5% uranyl acetate for approximately five minutes at room temperature. Subsequently, the leukocyte sample was washed four times with double distilled water.

Experimental Results:

As can be seen in Figure 11, the average number of lipid bodies per neutrophil cell of a leukocyte sample is approximately 0.83 with a standard deviation of 0.0314 in the control group and approximately 6.68 with a standard deviation of 1.701 in the ApoE knockout mice group. It can be seen that the average number of lipid bodies per neutrophil cell is significantly higher in the ApoE knockout mice group than in the control group. Correspondently, the average area of lipid bodies per neutrophil cell of a leukocyte sample is approximately 0.06 μm^2 with a standard deviation of 0.0043 μm^2 in the control group and approximately 0.671 μm^2 with a standard deviation of 0.255 μm^2 in the ApoE knockout mice group. It can be seen that the average lipid body area per neutrophil cell is significantly higher in the ApoE knockout mice group than in the control group (Figure 12).

Turning to Fig. 13, which is a statistical distribution of the average number of lipid bodies per neutrophil cell vs. the average lipid body area per neutrophil cell of leukocyte samples obtained from control mice and atherosclerotic ApoE knockout mice, it can be seen that the statistical distribution of the ApoE knockout mice group differs from the statistical distribution of the control group.

It is noted the in Examples 1-3 hereinabove the measured lipid body area is a surface confined within the great circle of the sphere-like shape defining the lipid body.

It will be appreciated by persons skilled in the art that the present invention is not limited by what has been particularly shown and described herein above. Rather the scope of the present invention includes both combinations and subcombinations of the various features described hereinabove as well as variations and modifications which would occur to persons skilled in the art upon reading the specifications and which are not in the prior art.

CLAIMS

What is claimed is:

1 A method for detecting a metabolic malady in a subject, comprising:

introducing a blood sample containing a leukocyte into an examination system, said blood sample being derived from the subject; and

analyzing a quantitative feature of a lipid body in said leukocyte, wherein if a value of said quantitative feature of said lipid body in said leukocyte of said blood sample derived from the subject is significantly different than a threshold, the subject is detected as having a metabolic malady.

2. A method for early detection of a metabolic malady comprising:

introducing a blood sample containing a leukocyte into an examination system, said blood sample being derived from a subject; and

analyzing a quantitative feature of a lipid body in said leukocyte, wherein if a value of said quantitative feature of said lipid body in said leukocyte of said blood sample derived from the subject is significantly different than a threshold, the subject is detected as having a susceptibility for developing a metabolic malady.

3. A method for monitoring a metabolic malady comprising:

introducing a blood sample containing a leukocyte into an examination system, said blood sample being derived from a subject; and

analyzing a quantitative feature of a lipid body in said leukocyte by evaluating if a value of said quantitative feature of said lipid body in said leukocyte of said blood sample derived from the subject is significantly different than a threshold, thereby monitoring a metabolic malady.

4. A method according to any one of claims 1 - 3, wherein said threshold is a threshold of a quantitative feature of a lipid body in a leukocyte of a blood sample derived from a control.

5. A method according to any one of claims 1 - 4, wherein said threshold is a threshold of a quantitative feature of a lipid body in a leukocyte of a blood sample derived from the subject following an administrated procedure.

6. A method according to claim 5, wherein said administrated procedure is physical activity, nutrient supplementation, a progression of time, a medical treatment, a diet or a fast.

7. A method according to any one of claims 1 - 6, wherein said quantitative feature is a lipid body area, a total lipid body area, an average lipid body area, a median lipid body area, a maximal lipid body area in at least one leukocyte, a minimal lipid body area in at least one leukocyte, a lipid body content, an average lipid body content, a median lipid body content, a maximal lipid body content in at least one leukocyte, a minimal lipid body content in at least one leukocyte, a number of lipid bodies, a total number of lipid bodies, an average number of lipid bodies, a median number of lipid bodies, a maximal number of lipid bodies in at least one leukocyte, or a minimal number of lipid bodies in at least one leukocyte, a lipid body weight, a lipid body volume, a lipid body size, a lipid body diameter, a lipid body radius or a combination thereof.

8. A method according to any one of claims 1 – 7, wherein analyzing comprises calculating said value of said quantitative feature of said lipid body in said leukocyte of said blood sample derived from said subject and comparing said value with said threshold.

9. A method according to any one of claims 1 - 8, wherein significantly different is a difference of approximately at least 30%.

10. A method according to any one of claims 1 – 9, wherein significantly different is a difference of approximately at least a factor of 4.

11. A method for assessing the efficacy of a ligand for metabolic malady prevention or treatment comprising:

obtaining a blood sample containing a leukocyte from a subject with a metabolic malady, following administration of said ligand to said subject;

introducing said blood sample into an examination system; and

analyzing a quantitative feature of a lipid body in said leukocyte of said blood sample, wherein if a value of said quantitative feature of said lipid body in said leukocyte of said blood sample is lower than a value of a quantitative feature of a lipid body in a leukocyte of a blood sample of an untreated subject with a metabolic malady, said ligand being detected as efficacious for treatment or prevention of a metabolic malady.

12. A method according to any one of claims 1 – 11, wherein introducing a blood sample containing a leukocyte into an examination system comprises inserting said sample into a sample container.

13. A method according to any one of claims 1 - 12, wherein said lipid body is in a neutrophil population, eosinophil population, basophil population, lymphocyte population, monocyte population, or a macrophage population of said blood sample.

14. A method according to any one of claims 1 - 13, wherein said subject is a human subject.

15. A method according to any one of claims 1 - 14, wherein said subject is an animal.

16. A method according to any one of claims 1 - 15, wherein said metabolic malady is a metabolic disease, a metabolic disorder, atherosclerosis, glucose intolerance, type II diabetes, a metabolic syndrome, insulin resistance, pre-diabetes, lipotoxicity, fatty liver, steatohepatitis, steatosis, obesity, a stroke, a cardio-vascular disease, hyperlipidemia, a metabolic malady complication, a diabetes complication, diabetic retinopathy or diabetic nephropathy.

17. A method according to any one of claims 1 – 16, wherein said examination system is a SEM.

18. A method according to any one of claims 1 – 17, wherein said examination system is a microscope, FACS or ELISA.

19. A kit for metabolic malady analysis of a subject, the kit comprising:

a sample container for inserting a blood sample containing a leukocyte therein; and

data for analysis of a quantitative feature of a lipid body in said leukocyte so as to detect the presence of the metabolic malady.

20. A kit according to claim 19, wherein said data is comprised in a paper or an electronic sheet.

21. A kit according to either of claims 19 and 20, wherein said data comprises a threshold of said quantitative feature of said lipid body, the metabolic malady analysis being performed by comparing said threshold with a value of a quantitative feature of a lipid body.

22. A kit according to any one of claims 19 - 21, wherein said quantitative feature is a lipid body area, a total lipid body area, an average lipid body area, a median lipid body area, a maximal lipid body area in at least one leukocyte, a minimal lipid body area in at least one leukocyte, a lipid body content, an average lipid body content, a median lipid body content, a maximal lipid body content in at least one leukocyte, a minimal lipid body content in at least one leukocyte, a number of lipid bodies, a total number of lipid bodies, an average number of lipid bodies, a median number of lipid bodies, a maximal number of lipid bodies in at least one leukocyte, or a minimal number of lipid bodies in at least one leukocyte, a lipid body weight, a lipid body volume, a lipid body size, a lipid body diameter, a lipid body radius or a combination thereof.

23. A kit according to any one of claims 19 - 22, wherein said lipid body is in a neutrophil population, eosinophil population, basophil population, lymphocyte population, monocyte population or a macrophage population of said blood sample.

24. A kit according to any one of claims 19 - 23, wherein said metabolic malady is a metabolic disease, a metabolic disorder, atherosclerosis, glucose intolerance, type II diabetes, a metabolic syndrome, insulin resistance, pre-diabetes, lipotoxicity, fatty liver, steatohepatitis, steatosis, obesity, a stroke, a cardio-vascular disease, hyperlipidemia, a metabolic malady complication, a diabetes complication, diabetic retinopathy or diabetic nephropathy.

25. A kit according to any one of claims 19 – 24, comprising an examination system.

26. A kit according to claim 25, wherein said examination system is a SEM.

27. A kit according to any one of claims 19 – 26, wherein the metabolic malady analysis of the subject is for metabolic malady detection, metabolic malady diagnosis, early detection of a metabolic malady, early detection of a metabolic malady, assessing efficiency of a ligand for metabolic malady prevention or treatment, monitoring deterioration of a subject with a metabolic malady, or monitoring improvement of a subject with a metabolic malady.

28. A method comprising:

introducing a blood sample containing a leukocyte into an examination system, said blood sample being derived from a subject; and

analyzing a quantitative feature of a lipid body in said leukocyte.

29. A method for metrology of a quantitative feature of a lipid body in a leukocyte sample comprising:

introducing a blood sample containing a leukocyte into an examination system, said blood sample being derived from a subject; and

analyzing the quantitative feature of the lipid body in the leukocyte.

FIG. 1A

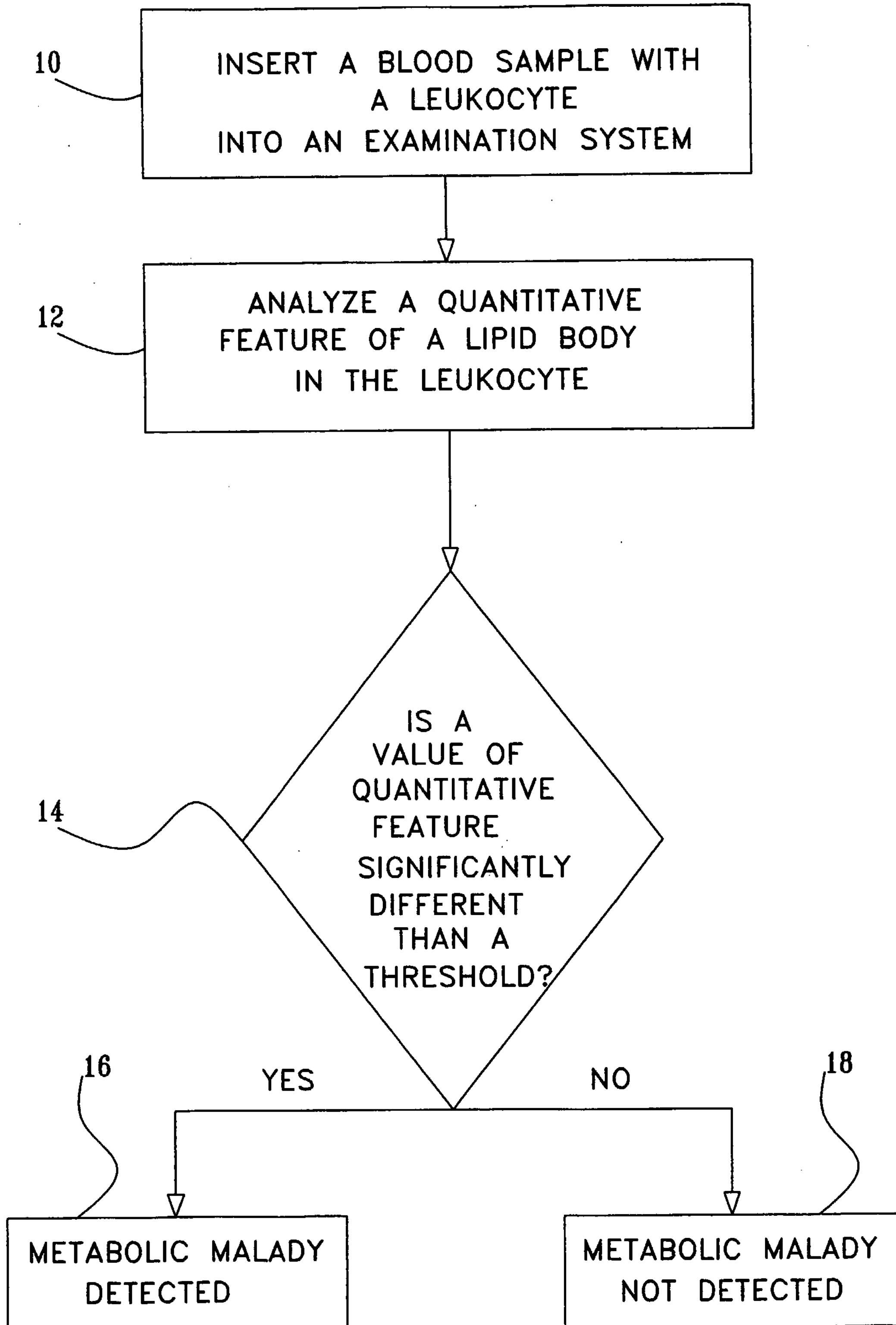

FIG. 1B

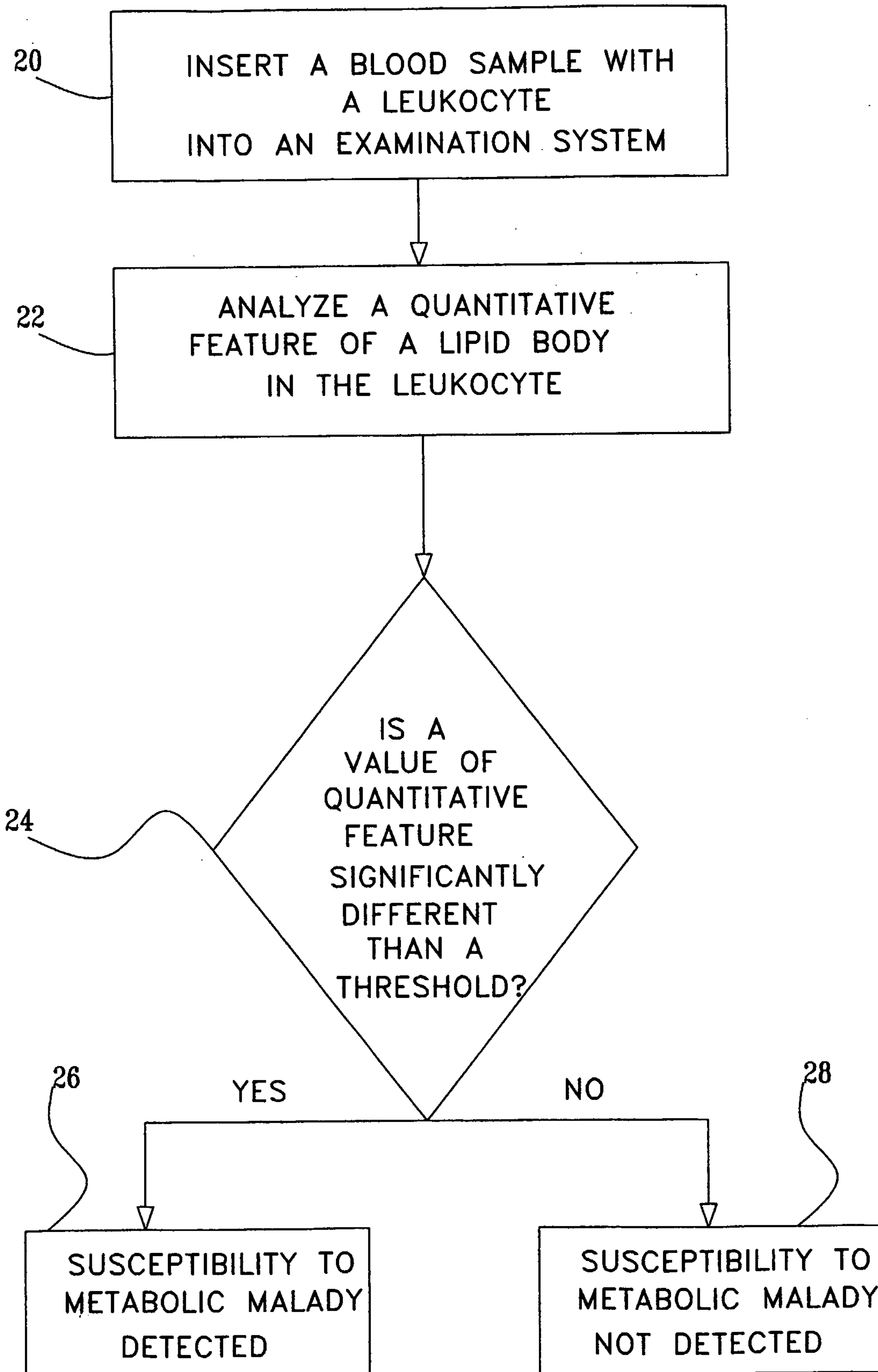

FIG. 1C

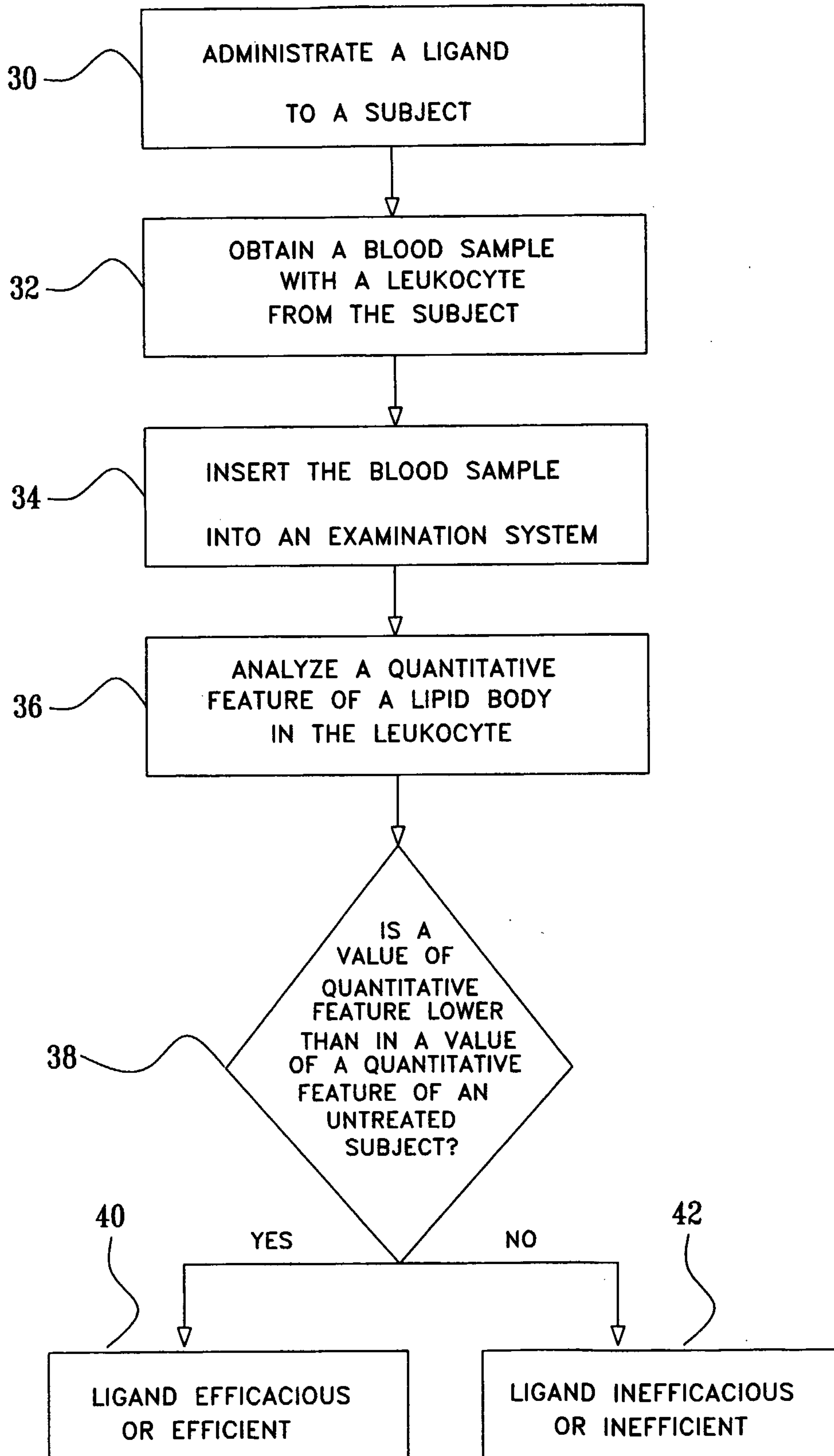

FIG. 1D

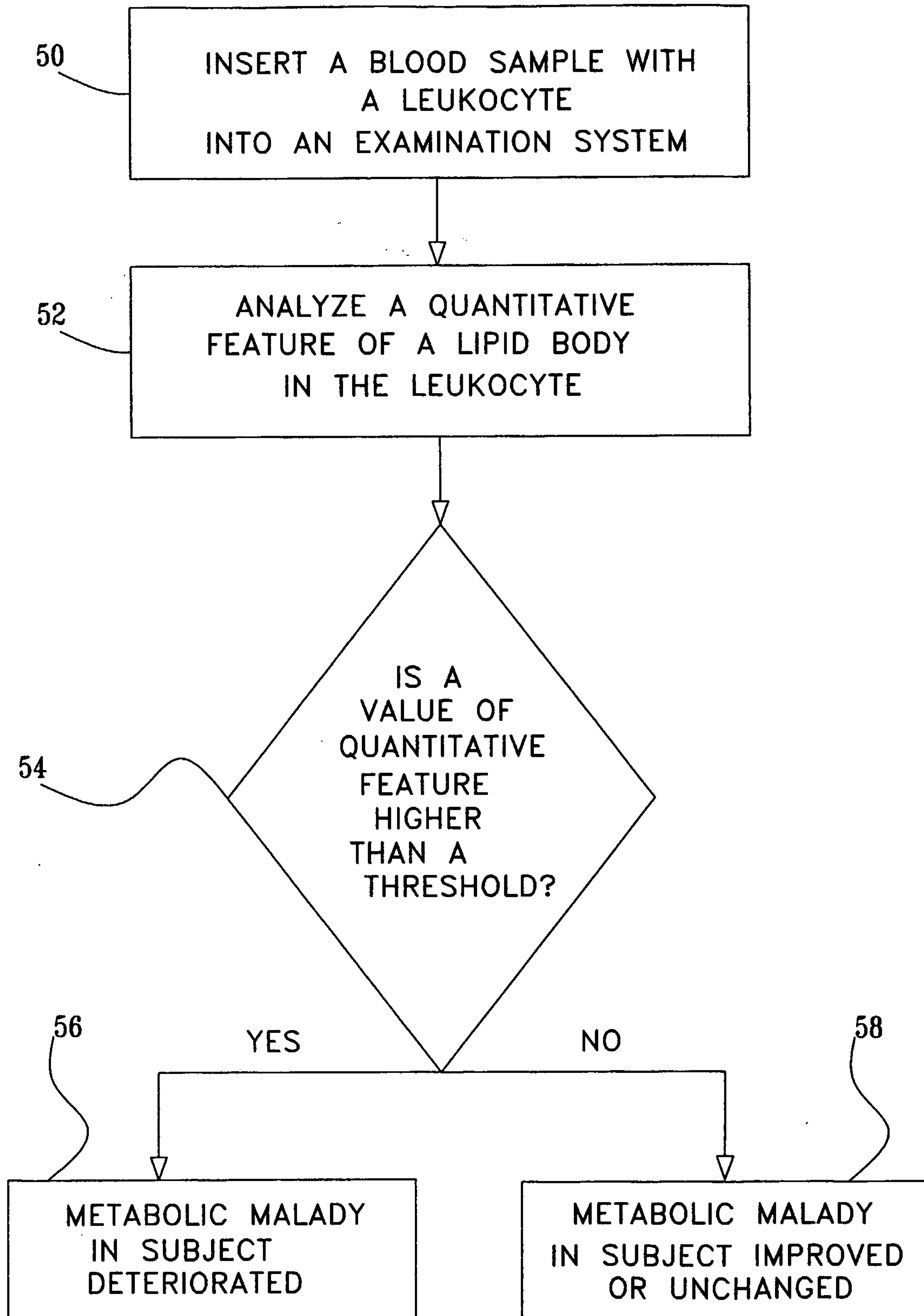

FIG. 2A

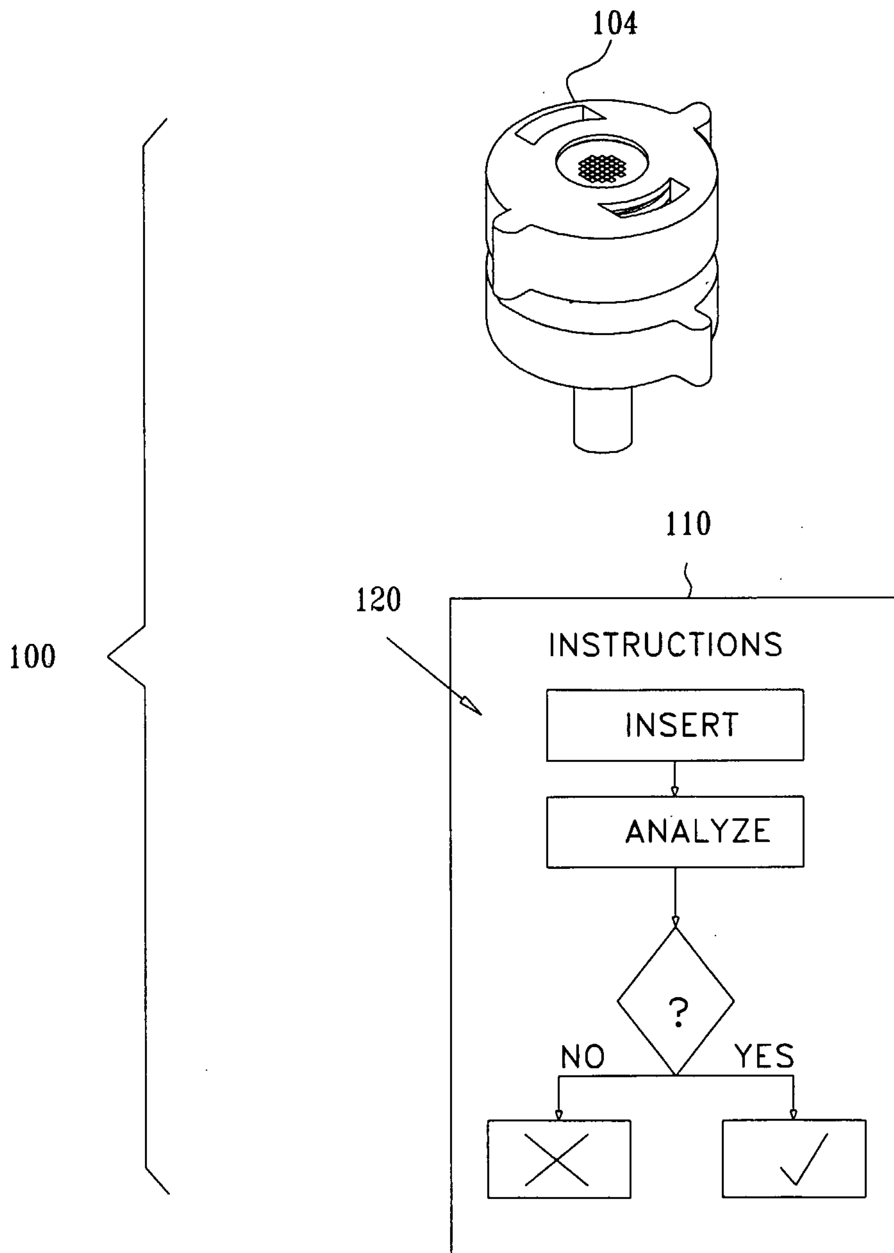

FIG. 2B

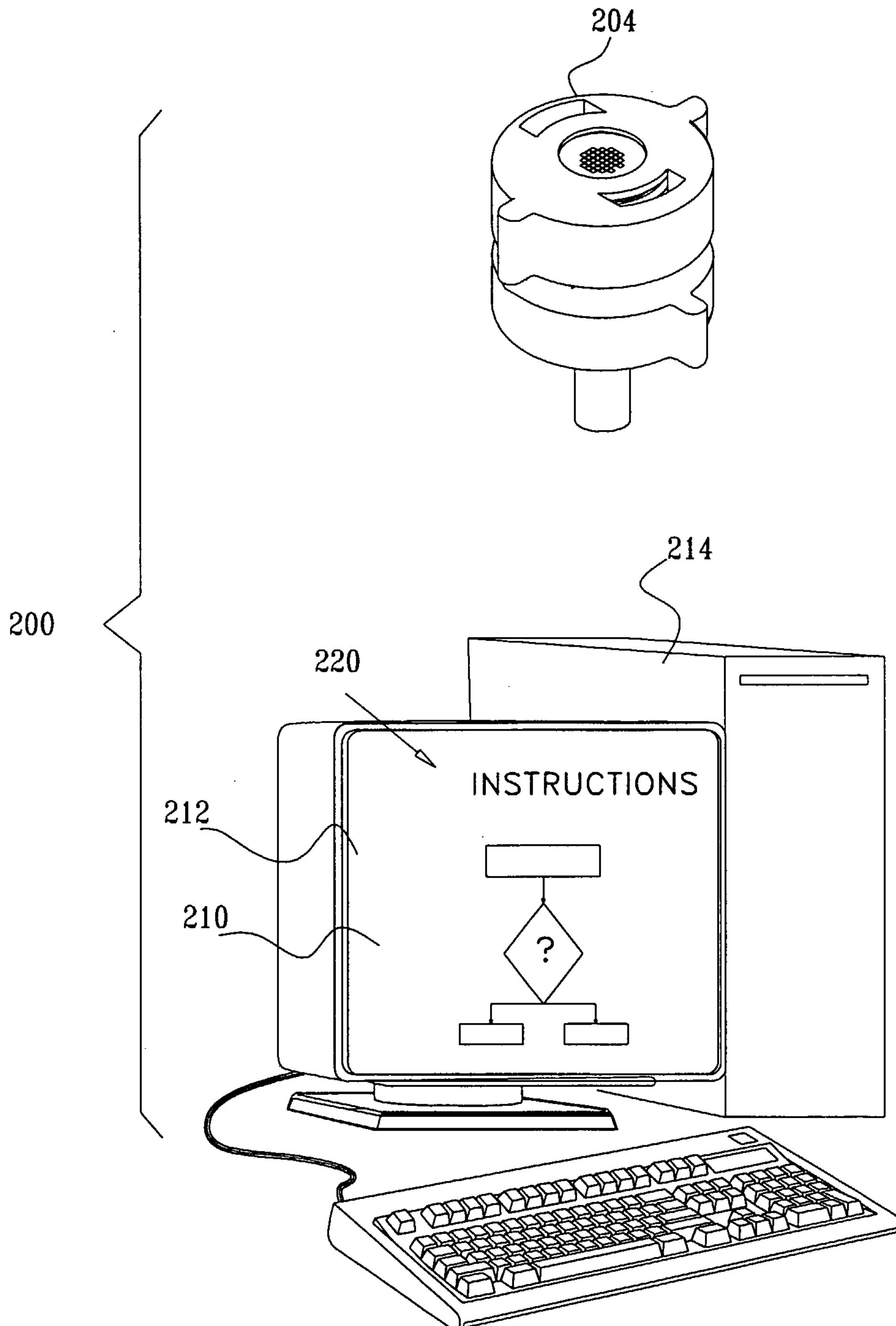

FIG. 3A

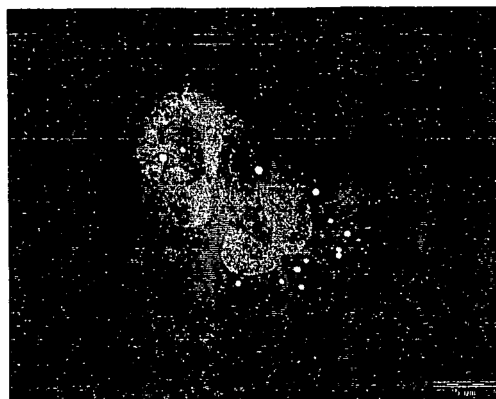

FIG. 3B

FIG. 4A

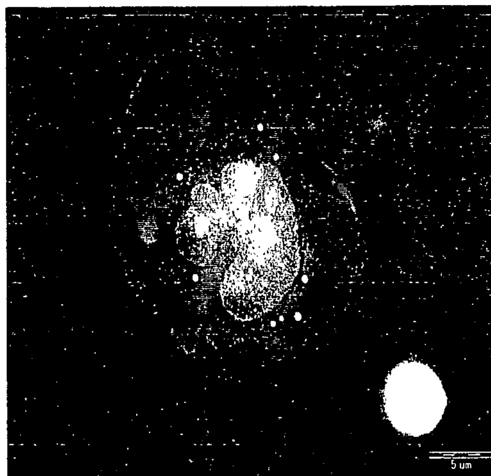

FIG. 4B

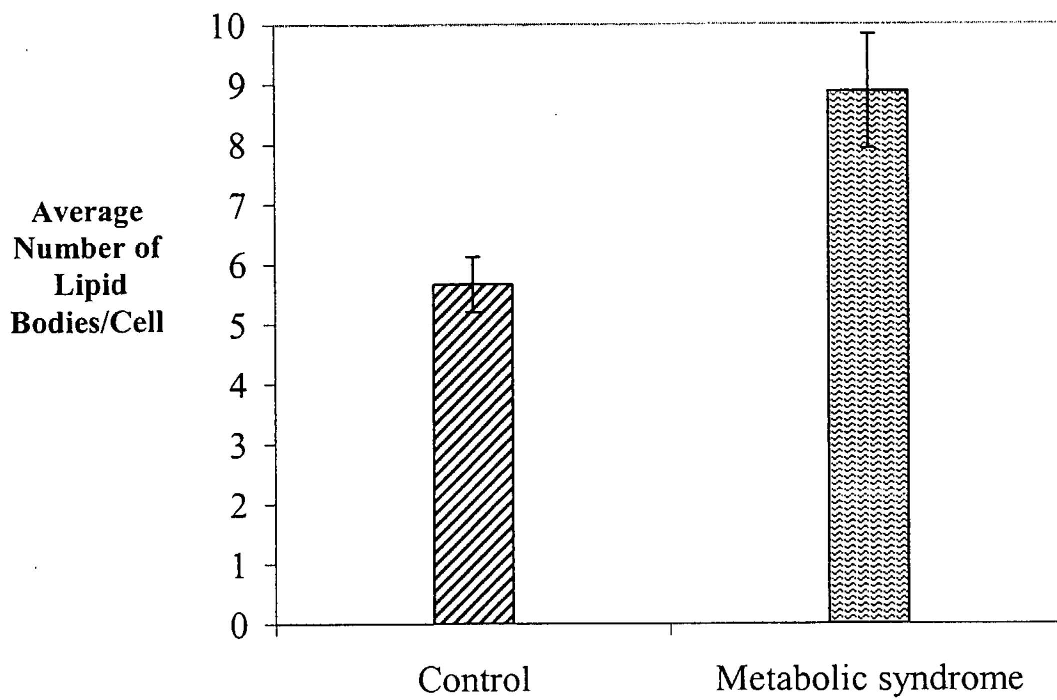

FIG. 5

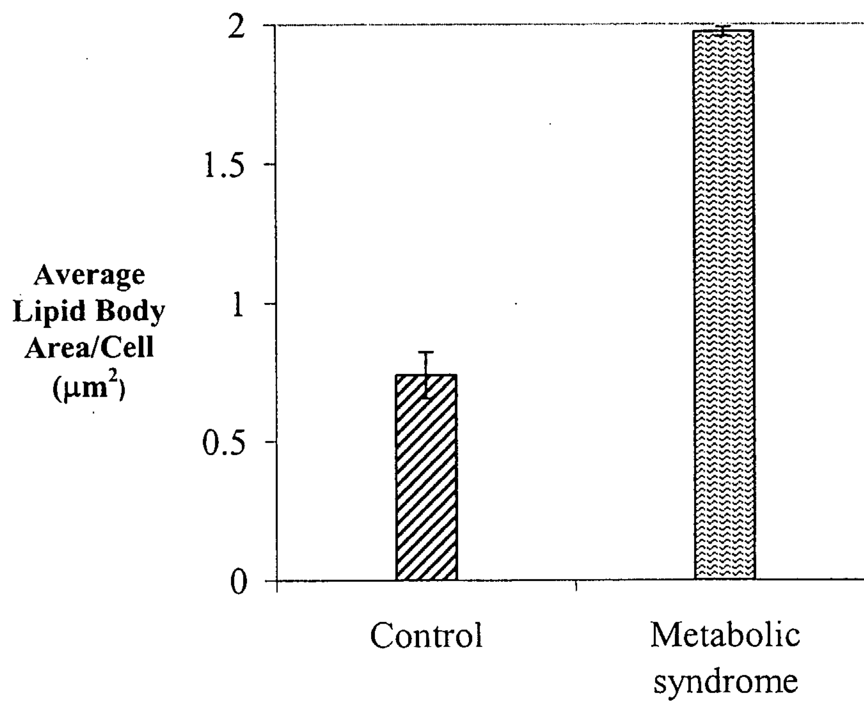

FIG. 6

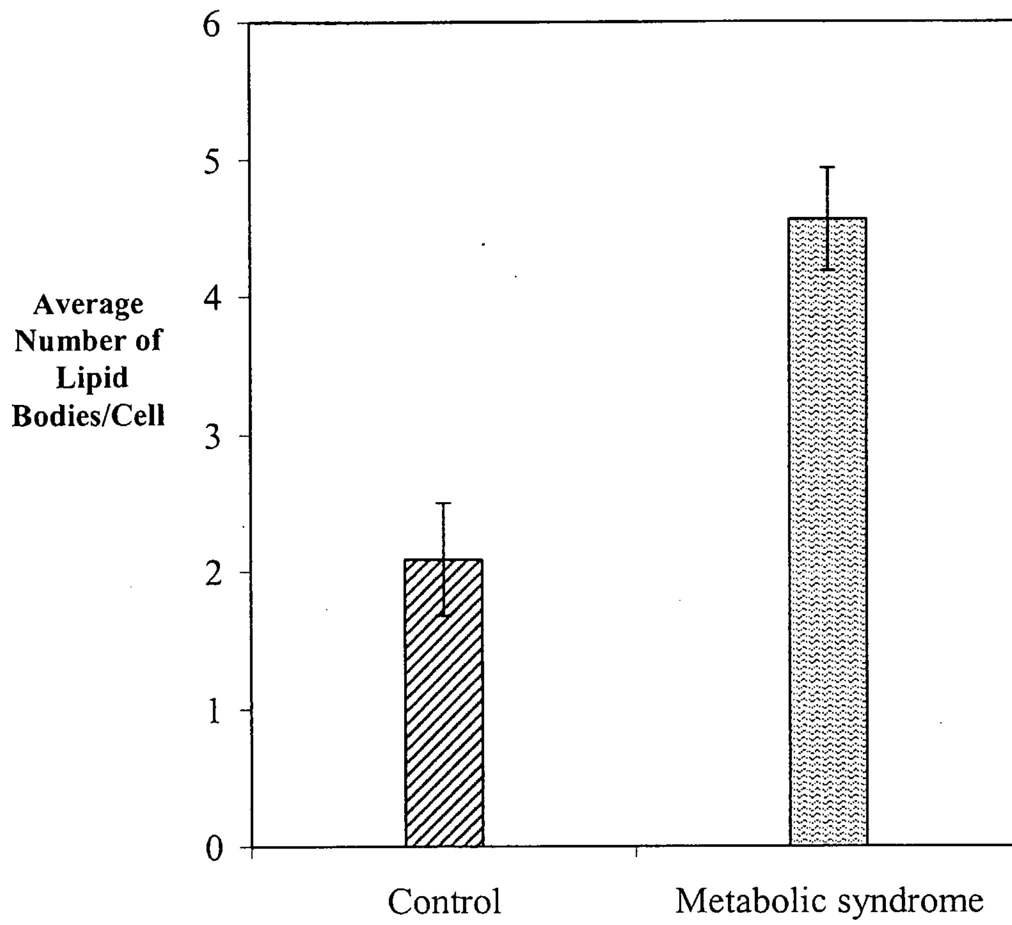

FIG. 7

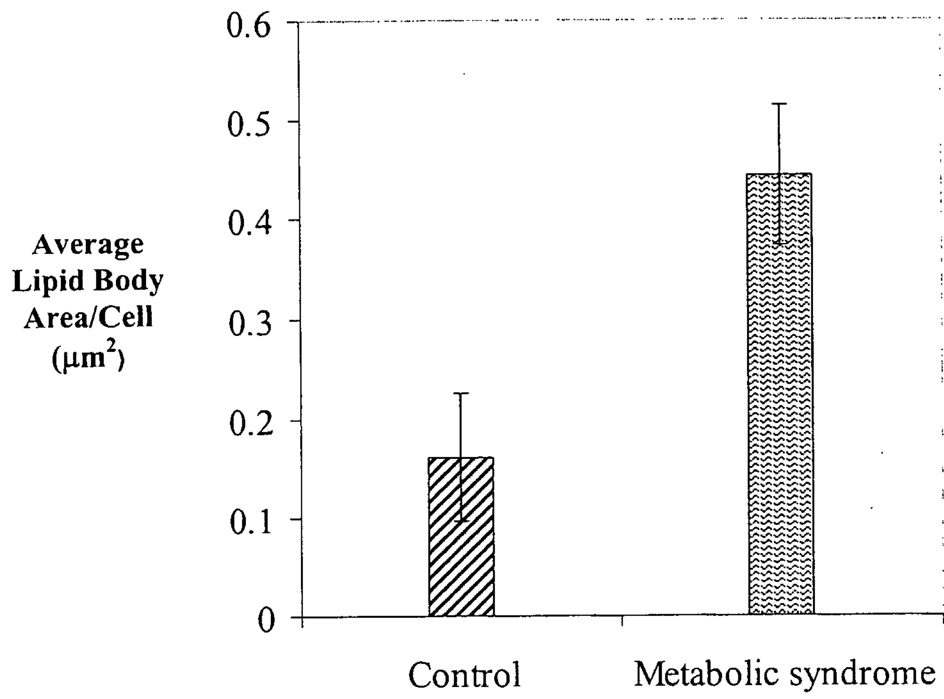

FIG. 8

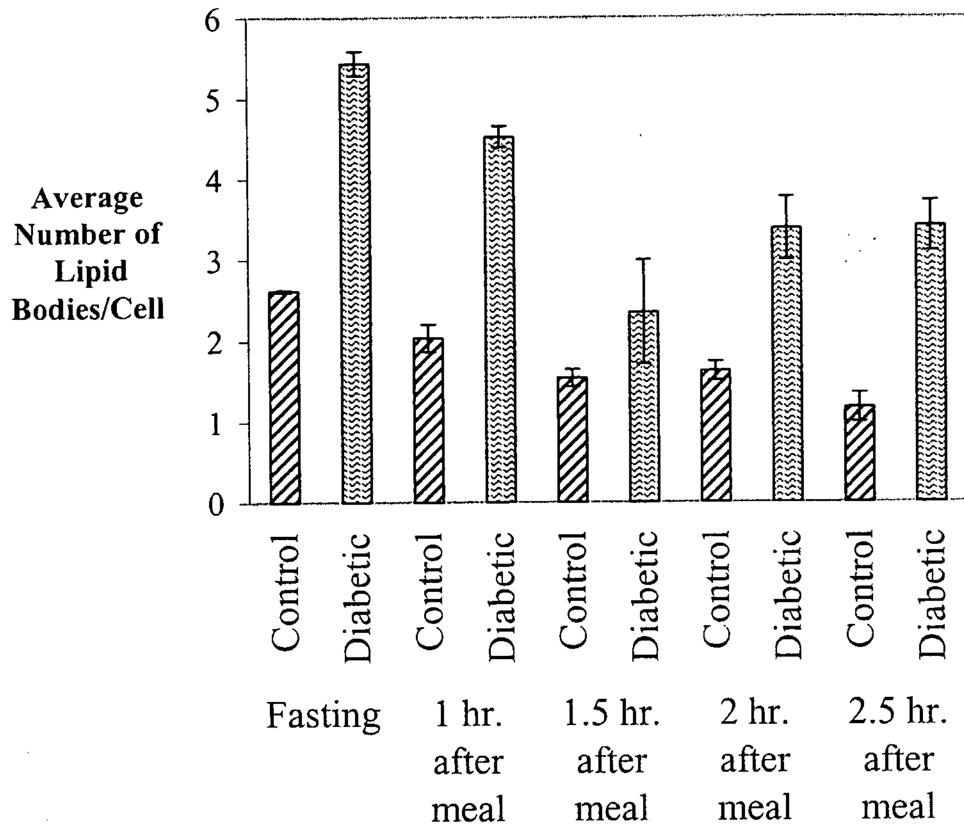

FIG. 9

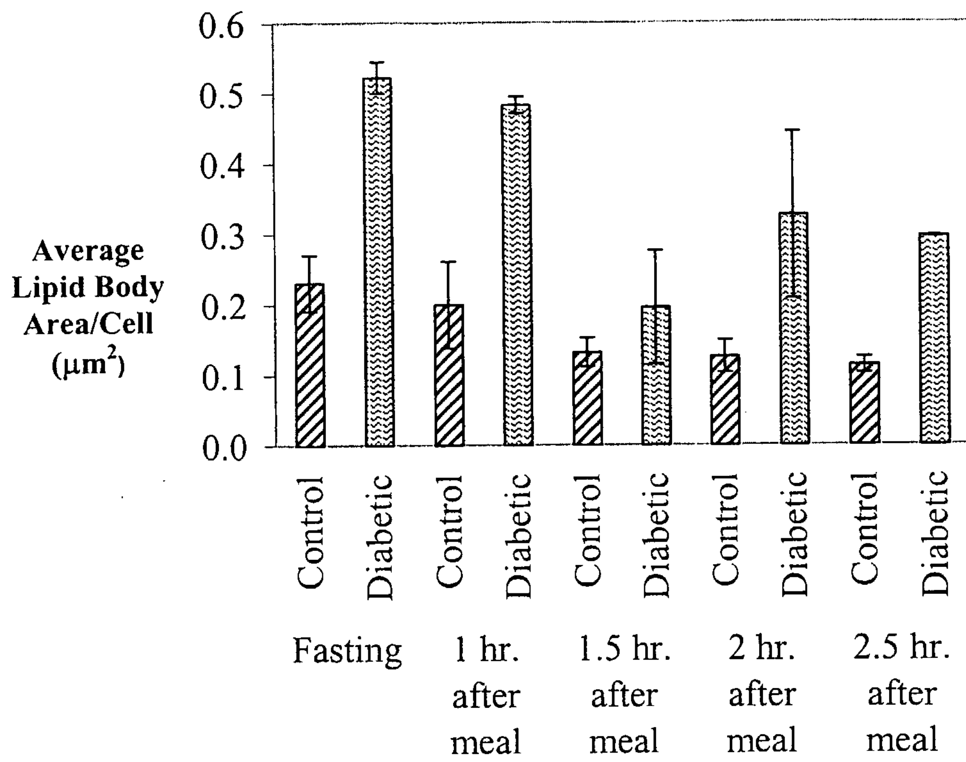

FIG. 10

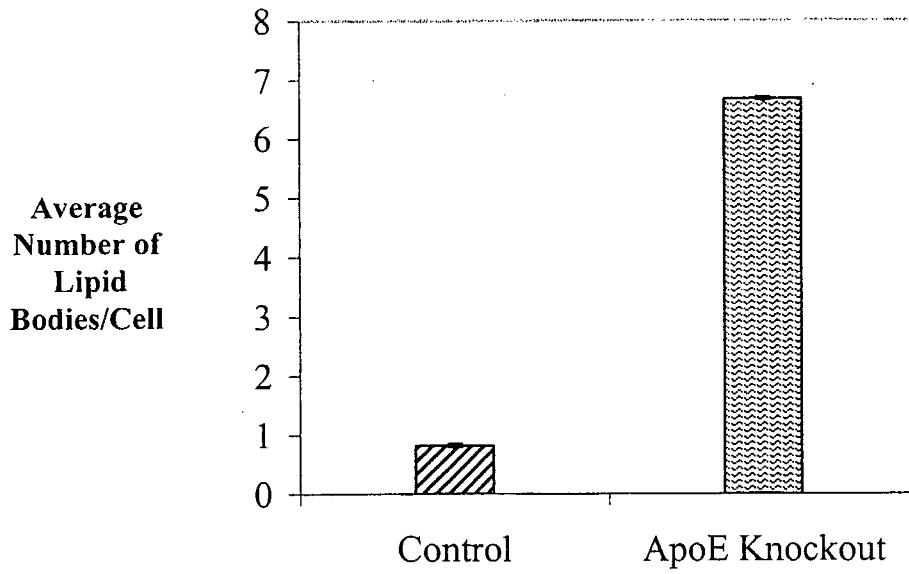

FIG. 11

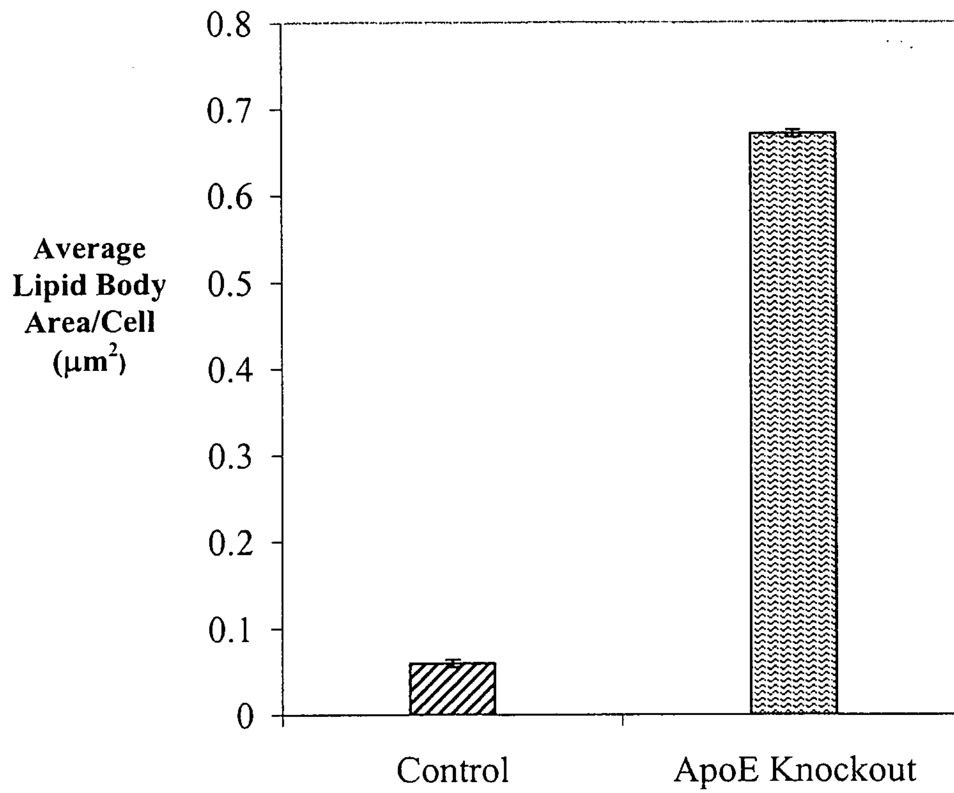

FIG. 12

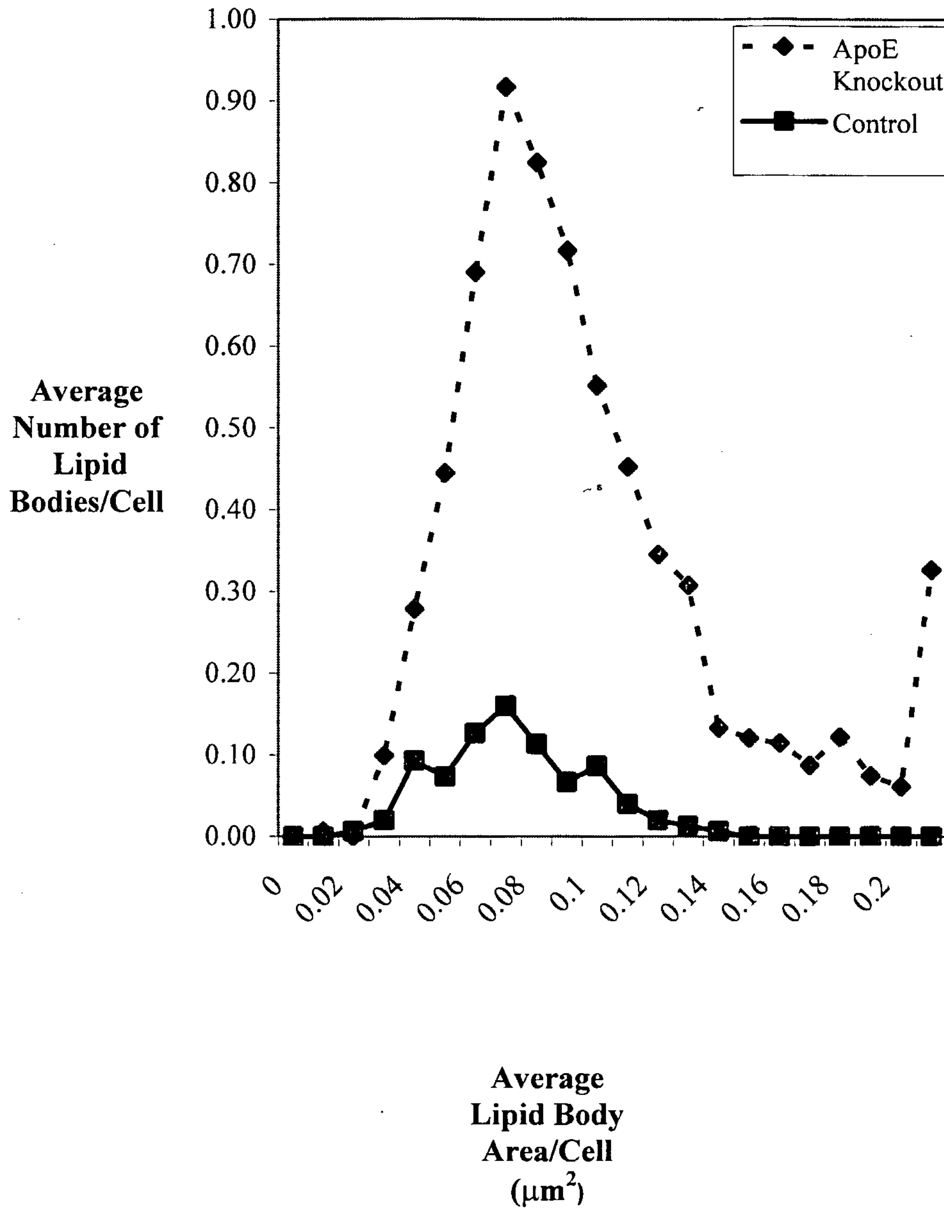

FIG. 13

patsnap

专利名称(译)	用于分析代谢疾病的装置和方法		
公开(公告)号	EP1812793A2	公开(公告)日	2007-08-01
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优先权	60/627157 2004-11-15 US 60/671541 2005-04-15 US		
其他公开文献	EP1812793A4		
外部链接	Espacenet		

摘要(译)

一种检测受试者代谢疾病的方法，包括将含有白细胞的血液样品引入检查系统，血液样品来自受试者，并分析白细胞中脂质体的定量特征，其中如果值来自受试者的血液样品的白细胞中脂质体的定量特征与阈值显着不同，该受试者被检测为具有代谢疾病。