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(54) **Title**: METHOD FOR MONITORING PHYSICAL TRAINING IN HEALTHY AND DISEASED INDIVIDUALS

(57) **Abstract**: The present patent application is concerned with the provision of means and methods for assessing the effects of physical training regimen in a subject. Specifically, the invention allows the assessment whether physical training induces ischemia and/or angiogenesis in a subject, whether it increases the subject's resistance to reperfusion injury and whether an excessive intensity of physical training causes cardiac damage.

Method for monitoring physical training in healthy and diseased individuals

The present patent application is concerned with the provision of means and methods for assessing the effects of physical training regimen in a subject. Specifically, the invention allows the assessment whether physical training induces ischemia and/or angiogenesis in a subject, whether it increases the subject's resistance to reperfusion injury and whether an excessive intensity of physical training causes cardiac damage.

Physical exercise has different objectives. In athletes the major purpose is to increase muscle growth either to improve endurance such as in marathon runners, triathlon athletes, cyclists or other sports requiring endurance. Other athletes require maximal force or speed only for a short period of time, as is the case in weight lifters, short track runners or the like. In a population with a sedentary lifestyle sports or physical exercise have become popular either to improve personal well being or to promote a healthy lifestyle and to reduce the risk of cardiovascular morbidity and mortality (Ekelund L.G. et al., N Engl J Med 1988, 319: 1379 – 84; Paffenberger R.S. et al., N Engl J. Med 1993, 328: 538 – 45). The extent and frequency of physical exercise varies significantly in non-professional sports and so do training regimens. Training regimens my include long distance walking, running or cycling or intensity training, where a high intensity phase is followed by a recovery phase and where the intensity phase can be increased. Such intensity training is typically done using a cycling ergometer and the recording of heart rate, maximal oxygen uptake and lactate determination. Physical training can be executed with or without artificial mechanical stimulation. Typical mechanical stimulation is the use of vibrators or electrical muscle stimulation, the latter being also used without physical activity. Exercise can be carried out under aerobic and anaerobic conditions. Physical activity can be monitored as described above by measuring heart rate, maximal oxygen uptake or lactate. In addition to this, the result of the training regimen, i.e. the improvement of physical activity, such as time needed for a specific distance, weight lifted and so on, can be used for monitoring the effect of a training regimen. If these parameters improve, the exercise program is believed to be successful. Exercise is, however, not without harm. In case of ischemia of the muscle, reperfusion injury might occur which damages the muscle. In animal models brief episodes of ischemia have been shown to reduce reperfusion injury

after prolonged ischemia in different animal models of cardiac ischemia and hind limb ischemia (Van Vuuren D., Lochner A., 2008, Cardiovascular J. of Africa, 19: 311- 320; A.E. et al J. Surg Res 2009, in press). Angiogenesis has been studied in animal models of physical exercise, using knock out rats. It was shown that PlGF does not contribute to exercise induced angiogenesis in rats (Lloyd P.G. et al., Am J Physiol Heart Circ Physiol 288: H759–H768, 2005). In human athletes exercise was shown to induce VEGF as well as endostatin under hypoxic conditions, the increase of both markers occurred under physiological (hypoxia) and mechanical (vibration) conditions (Suhr F. et al., 2007, J Appl. Physiol 103: 474–483). Such methods have, however, not been implemented in training programs of professional athletes or in non-professional sports.

In individuals with comorbidities and, specifically, coronary heart disease such training programs are even more complex as they do not only effect the skeletal muscle but also the heart muscle because physical training programs increase the heart rate and, consequently, the oxygen demand of the heart. In the case of coronary artery disease, this may result in cardiac ischemia. Cardiovascular comorbidities may be obvious, e.g. in case the patient has presented previously with acute coronary syndrome and been diagnosed with coronary artery disease e.g. by angiography. Said comorbidities may, however, be non-obvious, and the patient may not have experienced signs of CAD previously. In patients with myocardial infarction physical training programs have been established. These programs recommend aerobic exercise with 75% peak or ischemic heart rate for a duration of 20 minutes 3 times a week. (Thompson P.D., Braunwalds Heart Disease, chapter 46, p 1149 ff). In addition an exercise test is recommended before the training program is started.

The risk of physical training after acute coronary syndrome or myocardial infarction is reflected by algorithms that take into account whether or not the myocardial infarction was uncomplicated. In addition to this, risk stratification models have been implemented which include measurement of left ventricular function (LVEF) by echocardiography, arrhythmia, exercise capacity, hemodynamic response to exercise, clinical information as well as exercise induced cardiac ischemia based on symptoms and ECG-changes (Piotrowicz et al., 2008, 15: 481-487). The intensity of exercise in these patients is generally determined by the ventilatory oxygen consumption or the heart rate. The exercise training is generally performed at 60% to 70% VO_{2max} which corresponds to 70% to 80% maximum heart rate. VO_{2max} is determined before the patient enters the rehabilitation program. Since this approach determines the stress induced by the exercise rather than directly monitoring the

positive effect (or the negative effect) on the cardiovascular system, refined methods for adapting the exercise in cardiac rehabilitation programs are desirable.

For athletes in general, reliable methods for selecting the right extent and intensity of physical training have been lacking so far. If a muscle consumes more oxygen than can be supplied it switches to lactic acid fermentation as a means for generating energy. If the build-up of lactic acid reaches a certain level, lactic acid is released into the blood stream. The anaerobic threshold, i.e. the level of exercise where the aerobic metabolism fails to meet the energy requirements of the body, of an individual can thus be determined. Lactic acid can be detected in the muscle as well as in peripheral blood. Accumulation of lactic acid is an indicator for intensive exercise that induces ischemia. However, the accumulation of lactic acid depends on factors other than the anaerobic threshold. For example, the transport rate of lactate into the bloodstream is another important determinant of lactate concentration. Other methods for the optimization of physical training are the respiratory quotient and the heart rate. All of these methods have a common disadvantage: they can be used to determine the physical stress caused by exercise more or less reliably. However, they can not be used to determine the body's adaptation to the physical stress. Because the desired effect of physical training is the adaptive response of the body to the stress rather than the physical stress as such but rather, this is a serious drawback.

Troponins represent structural proteins of myocytes that are released upon myocyte death. Troponins have been used for the diagnosis of myocardial infarction (Reichlin et al., 2009, NEJM, 361: 858-867; Keller et al., 2009, NEJM, 361: 868-877). With the development of high sensitivity troponin tests persistent elevations of troponins have been noted that correlate with the extent of heart failure as with coronary artery disease (EP 07 114 174).

Cardiac troponin T is known as a biochemical in the context of myocardial infarction and coronary artery disease (Omland, 2010, J. Intern. Med.; 268: 207-217). Currently, it represents the "gold standard" for the diagnosis of this condition.

Soluble fms-like tyrosine kinase (sFlt-1) is a receptor for vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) (Kendall R, Thomas K., 1993, Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci USA; 90:10705-10709.)

GDF-15 has been shown in vitro and in animal models to reflect a response to reperfusion injury and to protect cardiomyocytes from reperfusion injury (Kempf et al., 2006, Circulation Research, 98: 351-360) and has also been found in vivo after STENT implantation (PCT/EP 2008/054580).

Thus, there is an urgent need for novel methods of monitoring the effects of physical training in sports medicine in general and in cardiovascular rehabilitation programs in particular. These methods should allow the direct monitoring of the physiological adaptation to exercise and/or exercise-induced damage rather than judging the effect just by monitoring the effort of the subject.

Therefore, the present invention relates to a method for assessing the effects of a physical training regimen in a subject based on the determination of the amounts of sFlt-1 and a cardiac troponin in a sample of the patient and the comparison of the determined amounts to reference amounts.

It is also provided a method for assessing the effects of a physical training regimen in a subject comprising the steps of

a. determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject; and
b. comparing the amounts determined in step a) to reference amounts;

whereby the effects of the physical training regimen in the subject are assessed.

Preferably, the method of the present invention comprises the steps of a) determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject; b) comparing the amounts determined in step a) to reference amounts; and c) assessing the effects of the physical training regimen in the subject.

Accordingly, the present invention relates to a method for assessing the effects of a physical training regimen in a subject comprising the steps of

a. determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject;
b. comparing the amounts determined in step a) to reference amounts; and

c. assessing the effects of the physical training regimen in the subject.

The method of the present invention is, preferably, an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above including sample pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually and/or assisted by automation. Preferably, steps (a), (b) and/or (c) may in total or in part be assisted by automation including suitable robotic and sensory equipment for the determination in step (a) and/or a computer-implemented comparison under steps (b) and/or (c).

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well known techniques and include, preferably, samples of blood, plasma, serum, or urine, more preferably, samples of blood, plasma or serum. Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as centrifugation or cell sorting. Preferably, cell-, tissue- or organ samples are obtained from those cells, tissues or organs which express or produce the peptides referred to herein.

Preferably, samples for the determination of different markers are taken at different time points. sFlt-1 is, preferably, determined in a sample taken within about 30 minutes after the cessation of exercise. More preferably, said sample is taken immediately after cessation of exercise. The term "immediately" refers, preferably to a time span of not more than about 5 minutes or not more than about 10 minutes. Cardiac troponins are, preferably, determined in samples taken not more than about 1 hour, not more than about 2 hours, not more than about 3 hours or not more than about 4 hours after the cessation of exercise. More preferably the sample for the determination of cardiac troponins is taken after about 3 hours after the cessation of exercise. Preferably, the individual had at least about 4 hours rest before commencing the training session.

In order to assess the success of a physical training regimen continuously, it is preferred to take samples in regular intervals as long as the individual practices the physical training regimen. Said intervals are, preferably, about 2 weeks, about 3 weeks, about 4 weeks, about 8 weeks or about 10 weeks. A first sample is, preferably, taken before the physical training regimen is initiated in order to support the selection of a suitable physical training regimen for the individual.

Thus, the present invention also relates to a method for monitoring the effects of a physical training regimen in a subject comprising the repeated repetition of assessing the effects of physical training in a subject as described above.

The method of the present invention may be combined with the known methods for monitoring physical training described in the introduction.

The subject is, preferably, a human. In one embodiment of the invention, the subject is not in a physiological or pathophysiological state in which the amount/concentration of one or more of the markers sFLT-1, troponin T/I, PlGF and GDF-15 used in the context of the present invention is elevated. "Elevated" means higher than in the same subject not being in the particular physiological or pathophysiological state. In general, these physiological or pathophysiological states are known to the person skilled in the art. E.g. it is known that individuals with an impaired kidney function have elevated troponin levels and may have elevated levels of at least one of the other markers. It is also known that individuals suffering from coronary artery disease CAD have elevated levels of troponins and/or PlGF as a consequence of ischemia/necrosis. Accordingly, in this embodiment, the subject preferably has a normal kidney function as indicated by a glomerular filtration rate of more than 60 ml per minute and 1.73 m^2 of body surface and/or by a serum creatinine value of less than 1.3 mg/dl. Preferably, the subject undergoes physical training. Preferably In this embodiment, the individual is healthy with respect to diseases or disorders other than those cited in the context of the present invention that cause an increase of sFlT-1 and/or of PlGF. In particular, the individual is not pregnant (if the individual is a female individual). Preferably, the individual does not suffer from coronary artery disease. More preferably, the individual does not suffer from acute coronary syndromes, chronic inflammatory conditions and acute inflammatory conditions. The term "acute coronary syndromes" refers to myocardial infarction and unstable angina pectoris.

In another embodiment of the invention, the subject is in a physiological or pathophysiological state in which the amount/concentration of one or more of the markers sFLT-1, troponin T/I, PlGF and GDF-15 used in the context of the present invention is elevated. "Elevated" means higher than in the same subject not being in the particular physiological or pathophysiological state. In general, these physiological or pathophysiological states are known to the person skilled in the art. E.g. it is known that individuals with an impaired kidney function have elevated troponin levels and may have elevated levels of at least one of the other markers. It is also known that individuals

suffering from coronary artery disease CAD have elevated levels of troponins and/or PlGF as a consequence of ischemia/necrosis. Accordingly, in this embodiment, the subject preferably does not have a normal kidney function as indicated by a glomerular filtration rate of more than 60 ml per minute and 1.73 m^2 of body surface and/or by a serum creatinine value of less than 1.3 mg/dl. Preferably, the subject does not undergo physical training. Preferably In this embodiment, the individual is not healthy with respect to diseases or disorders other than those cited in the context of the present invention that cause an increase of sFlT-1 and/or of PlGF. In particular, the individual is pregnant (if the individual is a female individual). More preferably, the individual suffers from coronary artery disease. Even more preferably, the individual suffers from acute coronary syndromes, chronic inflammatory conditions and acute inflammatory conditions. The term "acute coronary syndromes" refers to myocardial infarction and unstable angina pectoris.

The subject may be a healthy subject. The subject may also suffer from coronary artery disease. Coronary artery disease (CAD) is most commonly caused by obstruction of the coronary arteries by atheromatous plaques (stenosis) resulting in ischemia and/or necrosis.

The degree/severity of a stenosis can be determined by coronary angiography or single-photon emission computed tomography (SPECT). While it is generally accepted that a stenosis of more than 60% of the luminal diameter is hemodynamically significant in that it may be responsible for a reduction in exercise-induced myocardial blood flow that causes angina and ischemia, it is not established how significant obstructions of lower severity (e.g. 30%, 40% or 50% diameter stenosis) are in CAD. Even though the functional significance of an approximately 50% diameter stenosis is less well established, the said 50% diameter stenosis is often a reference in the classification of CAD, in particular in the definition of a so-called “vessel disease”..

The vessel disease can be a one-vessel, two-vessel or three-vessel diseases, or a multi-vessel disease. Coronary vessels are known to the person skilled in the art. In the context of the present invention, the term “coronary vessels” comprises the (three) large coronary vessels as well as the medium size vessels and the small size vessels connected thereto. Thus, in the context of the present invention, the coronary vessel disease may, for example, occur as a macroangiopathy affecting the large coronary vessels, but also as a combinated macro- and microangiopathy affecting not only the large, but also the medium and small size coronary vessels.

Coronary artery disease can be subclassified into 1-, 2- or 3-vessel diseases depending if 1, 2 or 3 of the large coronary vessels are occluded, whereby stenosis of more than 50% should occur per vessel.

The term "physical training" as used in the present patent application refers to any kind of exercise that aims at increasing the physical capabilities of a subject. More preferably, the physical training aims at least at one of the following effects: increase of the strength of the skeletal muscles, increase of the strength endurance of the skeletal muscles, increase of the endurance of the skeletal muscles and increase of the endurance of the cardiovascular system.

In a preferred embodiment of the present invention the physical training aims at increasing the mass, strength or strength endurance of the skeletal muscles. In the context of the present application this type of physical training is referred to as “muscle-oriented training”. Subjects practicing muscle-oriented training are, typically, athletes and patients undergoing rehabilitation programs. Said rehabilitation programs aim, preferably, at the recovery after musculoskeletal injury, at the mitigation or prevention of musculoskeletal disorders such as lower back pain or at the recovery after stroke. Subjects who have an increased risk of suffering from acute cardiovascular events and undergo muscle-oriented training have to be monitored with special care because the physical training may precipitate acute cardiovascular events.

In another preferred embodiment of the present invention the physical training aims at increasing the endurance of the cardiovascular system. This type of physical training is referred to as “endurance training”. Subjects practicing endurance training are, typically, athletes and patients undergoing cardiovascular rehabilitation. Since patients in cardiovascular rehabilitation programs have an increased risk of suffering from a recurrent acute cardiovascular event precipitated by the endurance training, these patients have to be monitored closely.

For maximal success physical training has to be adapted to the desired aim. First of all, exercises have to be selected that affect the desired muscles and or/the cardiovascular system. For example, push ups can be used to increase the strength or strength endurance of the Triceps brachii, the Pectoralis major and the Pectoralis minor. Suitable exercises that increase the endurance of the cardiovascular system in general are, for example, walking, running, cycling or swimming.

In addition to the kind of exercise, the extent and the intensity of the physical training have to be chosen carefully. The extent of the training is, preferably, measured by the duration of a specific exercise, such as running for a defined amount of time, or by the number of repeats of a specific exercise, e.g. the number of push-ups. The intensity of training is, preferably, characterized by the effort required per unit of time, e.g. the speed of running or cycling, or the effort per repeat, e.g. the weight lifted in weight lifting. Preferred measures of the intensity of training are, preferably, the pulse rate during exercise, the oxygen consumption during exercise and/or the concentration of lactate in the peripheral blood. Optimal results are, typically, achieved if the extent and intensity of training are varied within one session of training or between different sessions of training. It is often advantageous to alternate between phases of high extent and intensity of training and recovery periods.

The combination of the specific exercises and the intensity and extent of physical training is referred to as "physical training regimen". Preferably, a physical training regimen defines the type of exercise, the intensity and the extent of exercise over a defined period of time. Preferably, for a short term regimen said period of time is the duration of one training session, more preferably it is about 1 hour, about 2 hours, about 3 hours or about 4 hours. For a medium term regimen said period is, preferably, about 1 week, about 2 weeks, about 3 weeks or about 4 weeks. For a long term regimen said period is, preferably, about 1 month, about 2 months, about 3 months or about 6 months.

Preferably, the training regimen is interval training. Interval training is characterized by alternating within one training session phases of high intensity of physical training close to the maximum exertion and recovery periods of lower intensity. The duration of the recovery phases is chosen to allow only a partial recovery. As the phases of high intensity are above the anaerobic threshold, the myocardium and the affected skeletal muscles experience periods of ischemia. This effect is, in principle, desired because it induces angiogenesis and improved protection of the myocardium against reperfusion injury. However, if ischemia exceeds certain levels, it may cause irreversible damage to the myocardium.

In another preferred embodiment of the present invention the physical training is electrical muscle stimulation (EMS). EMS stimulates one or more muscles by the application of electric impulses that mimic action potentials and, thus, induce the contraction of the affected muscles. The electrical current is delivered by electrodes that are applied to skin

close to the muscles that shall be stimulated. EMS is used in a therapeutic setting to prevent atrophy of unused muscles e.g. after musculoskeletal injuries. It is also occasionally applied to supplement the training of athletes. For the home-market consumer garments with integrated EMS circuitry are available, e.g. for the stimulation of the abdominal muscles. As with other types of physical training, the extent and intensity of EMS have to be carefully chosen in order to avoid damage due to ischemia while still inducing the desired effects of training.

In yet another preferred embodiment of the present invention the subject is an athlete undergoing physical training. Preferably, said physical training reaches an intensity that leads to episodes of ischemia in at least parts of the myocardium. Thus, any performance-oriented physical training regimen is suitable for monitoring according to the method of the present invention. Thus, the method of the present invention is not only suitable for professional athletes but also to amateurs who practice competitive sports. According to the present invention the specific type of exercise does not matter, as long as the aforementioned criteria are fulfilled.

In another preferred embodiment of the present invention the subject is a patient who is at increased risk of suffering from acute coronary syndromes (ACS). The term "acute coronary syndromes" refers to myocardial infarction and unstable angina pectoris. An increased risk of acute coronary syndromes is, typically, the consequence of coronary artery disease, high blood pressure, obesity, tobacco smoking, hypercholesterolemia, stress and/or diabetes.

Patients at increased risk of acute cardiovascular events are, preferably, identified by stress tests. Said stress tests can be performed on the ergometer or the stress of the cardiovascular system can be induced by drugs. The effects of the stress tests are, preferably, assessed by the symptoms of the patient (chest pain indicates increased risk), by an ECG (ST depression indicates increased risk), a thallium scan (reduced perfusion of the myocardium indicates increased risk) and echocardiogram (motility changes of the heart due to stunning indicate an increased risk). Computed tomography and positron emission tomography or a combination of both methods is another option for determining the risk of acute cardiovascular events in a patient.

Patients who already suffered from one or more myocardial infarctions are at an especially increased risk of suffering from a recurrent acute coronary syndrome. Therefore, in another

preferred embodiment of the present invention, the subject is a patient who has already suffered from at least one myocardial infarction in the past. More preferably, the patient is a subject participating in a cardiac rehabilitation program.

The term "cardiac rehabilitation program" refers to any systematic treatment of patients who survived an acute myocardial infarction. Cardiac rehabilitation programs aim at the secondary prevention of acute cardiovascular events as well as the improvement of the patient's quality of life. Preferably, cardiac rehabilitation programs as referred to in the present application comprise physical training.

Therefore, in an especially preferred embodiment of the present invention the physical training is endurance training of the cardiovascular system in patients at increased risk of acute coronary syndromes. It has been shown that endurance training reduces the risk of further acute coronary syndromes in patients who had already suffered from an acute coronary syndrome. Moreover, it generally reduces the cardiac risk in patients at increased risk of acute coronary syndromes. Since the myocardium in many of these patients is already damaged, it is especially important to select a physical training regimen that does not lead to further irreversible myocardial damage.

Thus, the term "assessing the effects of a physical training regimen in a subject" refers to the process of evaluating whether the physical training regimen leads to the desired effects described below while avoiding undesired effects. Based on said monitoring the physical training can be optimized. "Optimization" means that the intensity and duration of physical training are adapted to maximize the desired effects and to eliminate or at least to minimize the undesired effects. Desired effects of physical training are, preferably, an increased resistance against reperfusion injury and the induction of angiogenesis. Further desired effects depend on the type of training. In muscle-oriented training, the increase of muscle mass, muscle strength and/or muscle endurance is considered a desired effect. In endurance training, an increased endurance of the cardiovascular system is a desired effect. An undesired effect of physical training is, preferably, the induction of excessive or irreversible myocardial damage due to necrosis caused by ischemia. In the context of cardiovascular rehabilitation programs a successful physical training increases the resistance of the myocardium to reperfusion injury, improves the vascularization of the myocardium and, generally, decreases the patient's risk of suffering from acute coronary syndromes in the future.

Physical training is considered successful, i.e. the desired effects are attained, if it induces angiogenesis in the subject without causing permanent or excessive myocardial damage. Whether angiogenesis is induced primarily in the myocardium or in the skeletal muscles depends on the type of training. Muscle-oriented training primarily leads to angiogenesis in the skeletal muscles while endurance training primarily induces angiogenesis in the myocardium. Moreover, the physical training, preferably, increases the body`s resistance to reperfusion injury. Physical training is considered unsuccessful if it does not achieve the aforementioned aims. More preferably, physical training is considered unsuccessful if it causes excessive or permanent myocardial damage.

While the adaption of the physical training regimen is a measure that has effects in the medium and long term, patients at increased risk of acute cardiovascular events may require an immediate intervention if the method of the present invention indicates excessive myocardial damage. The administration of adiponectin (Goldstein et al., 2009, Nature Clinical Practice, 6: 27-35) or erythropoietin (Paschos et al., 2008, Int. J. Biol. Sci., 4: 161-168) has been shown to reduce reperfusion injury. Similarly, the induction of active protein phosphatase1-inhibitor-1 is a therapeutic option (Nicolaou et al., 2009, Circ. Res., 104: 1012-1020).

The term "soluble (s)FlT-1" as used herein refers to polypeptide which is a soluble form of the VEGF receptor Flt-1. It was identified in conditioned culture medium of human umbilical vein endothelial cells. The endogenous soluble Flt-1 (sFlt-1) receptor is chromatographically and immunologically similar to recombinant human sFlt-1 and binds VEGF with a comparable high affinity. Human sFlt-1 has been shown to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro. Preferably, sFlt-1 refers to human sFlt-1. More preferably, human sFlt-1 can be deduced from the amino acid sequence of Flt-1 as shown in Genebank accession number P17948, GI: 125361. An amino acid sequence for mouse sFlt-1 is shown in Genebank accession number BAA24499.1, GI: 2809071. Due to its binding to VEGF sFlt-1 inhibits VEGF-mediated angiogenesis.

The term "cardiac Troponin" refers to all Troponin isoforms expressed in cells of the heart and, preferably, the subendocardial cells. These isoforms are well characterized in the art as described, e.g., in Anderson 1995, Circulation Research, vol. 76, no. 4: 681-686 and Ferrieres 1998, Clinical Chemistry, 44: 487-493. Preferably, cardiac Troponin refers to Troponin T and/or Troponin I, and, most preferably, to Troponin T. It is to be understood that isoforms of Troponins may be determined in the method of the present invention

together, i.e. simultaneously or sequentially, or individually, i.e. without determining the other isoform at all. Amino acid sequences for human Troponin T and human Troponin I are disclosed in Anderson, loc cit and Ferrieres 1998, Clinical Chemistry, 44: 487-493.

Preferably the biological property of troponin I and its variant is the ability to inhibit actomyosin ATPase or to inhibit angiogenesis in vivo and in vitro, which may e.g. be detected based on the assay described by Moses et al. 1999 PNAS USA 96 (6): 2645-2650). Preferably the biological property of troponin T and its variant is the ability to form a complex with troponin C and I, to bind calcium ions or to bind to tropomyosin, preferably if present as a complex of troponin C, I and T or a complex formed by troponin C, troponin I and a variant of troponin T.

The term "Growth-Differentiation Factor-15" or "GDF-15" relates to a polypeptide being a member of the transforming growth factor (TGF)-β cytokine superfamily. The terms polypeptide, peptide and protein are used interchangeable throughout this specification. GDF-15 was originally cloned as macrophage-inhibitory cytokine-1 and later also identified as placental transforming growth factor-β, placental bone morphogenetic protein, non-steroidal anti-inflammatory drug-activated gene-1, and prostate-derived factor (Bootcov loc cit; Hromas, 1997 Biochim Biophys Acta 1354:40-44; Lawton 1997, Gene 203:17-26; Yokoyama-Kobayashi 1997, J Biochem (Tokyo), 122:622-626; Paralkar 1998, J Biol Chem 273:13760-13767). Similar to other TGF-β-related cytokines, GDF-15 is synthesized as an inactive precursor protein, which undergoes disulfide-linked homodimerization. Upon proteolytic cleavage of the N-terminal pro-peptide, GDF-15 is secreted as a ~28 kDa dimeric protein (Bauskin 2000, Embo J 19:2212-2220). Amino acid sequences for GDF-15 are disclosed in WO99/06445, WO00/70051, WO2005/113585, Bottner 1999, Gene 237: 105-111, Bootcov loc. cit, Tan loc. cit., Baek 2001, Mol Pharmacol 59: 901-908, Hromas loc cit, Paralkar loc cit, Morrish 1996, Placenta 17:431-441 or Yokoyama-Kobayashi loc cit. The biological functions of GDF-15 are highly dependent on cell type and context. In the context of cardiac disease, GDF-15 has been shown to protect cardiomyocytes from ischemia/reperfusion injury (Kempf, loc. cit.). In the context of cancer the overexpression of GDF-15 in vitro has been shown to increase the motility of cancer cell lines (Senapatis, S. et al., 2010, Oncogene, 29: 1293-1302).

The term "variant" encompasses also variants of the specific peptides of the present application. Such variants have at least the same essential biological and immunological properties as the specific cardiac Troponins, sFlt-1, GDF-15 or PlGF. In particular, they

share the same essential biological and immunological properties if they are detectable by the same specific assays referred to in this specification, e.g., by ELISA Assays using polyclonal or monoclonal antibodies specifically recognizing the said cardiac Troponins, sFlt-1, GDF-15 or PlGF. Most preferably, the biological property of troponin I is the ability to inhibit actomyosin ATPase or to inhibit angiogenesis in vivo and in vitro, which may e.g. be detected based on the assay described by Moses et al. 1999 PNAS USA 96 (6): 2645-2650). The biological property of sFlt-1 is, most preferably, to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro and the inhibition of VEGF-mediated angiogenesis. The biological property of GDF-15 is, most preferably, the increase the motility of cancer cell lines due to overexpression of GDF-15 (Senapatis, S. et al., 2010, Oncogene, 29: 1293-1302). Moreover, it is to be understood that a variant as referred to in accordance with the present invention shall have an amino acid sequence which differs due to at least one amino acid substitution, deletion and/or addition wherein the amino acid sequence of the variant is still, preferably, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical with the amino sequence of the specific cardiac Troponin or sFlt-1, preferably over the entire length of the specific peptide. Variants may be allelic variants or any other species specific homologs, paralogs, or orthologs. Moreover, the variants referred to herein include fragments of the specific cardiac Troponins, sFlt-1, GDF-15 or PlGF or the aforementioned types of variants as long as these fragments have the essential immunological and biological properties as referred to above. Preferably the biological property of troponin I and its variant is the ability to inhibit actomyosin ATPase or to inhibit angiogenesis in vivo and in vitro, which may e.g. be detected based on the assay described by Moses et al. 1999 PNAS USA 96 (6): 2645-2650). Preferably the biological property of troponin T and its variant is the ability to form a complex with troponin C and I, to bind calcium ions or to bind to tropomyosin, preferably if present as a complex of troponin C, I and T or a complex formed by troponin C, troponin I and a variant of troponin T. The endogenous soluble Flt-1 (sFlt-1) receptor binds VEGF with high affinity. Human sFlt-1 is shown to form a VEGF-stabilized complex with the extracellular domain of KDR/Flk-1 in vitro. PlGF has angiogenic activity in vitro and in vivo. For example, biochemical and functional characterization of PlGF derived from transfected COS-1 cells revealed that it is a glycosylated dimeric secreted protein able to stimulate endothelial cell growth in vitro (Maqlione, 1993, Oncogene 8(4): 925-31). Variants of GDF-15 can, preferably, be determined by the assay as described by Wollert et al. in Clinical Chemistry 53, No 2, 2007, p. 284-291.

Thus, the variants shall be recognizable by the aforementioned means or ligands used for determination of the amount of the cardiac troponins, sFlt-1, GDF-15 or PlGF. Such fragments may be, e.g., degradation products of the peptides of the present invention. Further included are variants which differ due to posttranslational modifications such as phosphorylation or myristylation.

In yet another preferred embodiment of the present invention the amount of PlGF or a variant thereof is determined in addition to sFlt-1 or a variant thereof and the cardiac troponin or a variant thereof and compared to a reference amount. It is also contemplated by the present invention to determine the amount of PlGF or a variant thereof in addition to the amounts of sFlt-1 or a variant thereof, a cardiac troponin or a variant thereof and GDF-15 or a variant thereof.

Preferably, the amount of PlGF or the variant thereof is determined in a sample taken not more than about 30 minutes, not more than about 60 minutes, not more than about 90 minutes or not more than about 120 minutes after cessation of exercise. More preferably, the sample for the determination of the amount of PlGF or the variant thereof is taken 60 minutes after the cessation of exercise.

The term "PlGF (Placental Growth Factor)" as used herein refers to a placenta derived growth factor which is a 149-amino-acid-long polypeptide and is highly homologous (53% identity) to the platelet-derived growth factor-like region of human vascular endothelial growth factor (VEGF). Like VEGF, PlGF has angiogenic activity in vitro and in vivo. For example, biochemical and functional characterization of PlGF derived from transfected COS-1 cells revealed that it is a glycosylated dimeric secreted protein able to stimulate endothelial cell growth in vitro (Maqlione, 1993, Oncogene 8(4): 925-31). Preferably, PlGF refers to human PlGF, more preferably, to human PlGF having an amino acid sequence as shown in Genebank accession number P49763, GI: 17380553.

In another preferred embodiment of the present invention the amount of GDF-15 or a variant thereof is determined in addition to sFlt-1 or a variant thereof and the cardiac troponin or a variant thereof variant thereof and compared to a reference amount.

Preferably, the amount of GDF-15 is determined in a sample taken not more than about 30 minutes, not more than about 60 minutes, not more than about 90 minutes or not more than about 120 minutes after cessation of exercise. More preferably, the sample for the

determination of the amount of GDF-15 or the variant thereof is taken 60 minutes after the cessation of exercise.

GDF-15 is a marker for reperfusion events. Increased levels of GDF-15 have been shown to protect the heart from reperfusion injury, thus amounts of GDF-15 higher than the reference amount indicate that the physical training regimen improves the subject´s resistance against reperfusion injury.

The term "reperfusion injury" refers to the damage to the structural and functional integrity of muscle cells, preferably myocardial cells, due to reestablishment of oxygen supply after a period of ischemia. It has been described that a significant part of muscular/myocardial damage in patients experiencing ischemia of the myocardium or skeletal muscles occurs after the end of ischemia rather than during ischemia. One possible mechanism for reperfusion injury is the progressive loss of ion homeostasis. Anatomically, a hypercontraction of myocardial cells can be observed during reperfusion. The cells finally die by necrosis.

The term “Growth-Differentiation Factor-15” or “GDF-15” relates to a polypeptide being a member of the transforming growth factor (TGF)-β cytokine superfamily. The terms polypeptide, peptide and protein are used interchangeable throughout this specification. GDF-15 was originally cloned as macrophage-inhibitory cytokine-1 and later also identified as placental transforming growth factor-β, placental bone morphogenetic protein, non-steroidal anti-inflammatory drug-activated gene-1, and prostate-derived factor (Bootcov loc cit; Hromas, 1997 Biochim Biophys Acta 1354:40-44; Lawton 1997, Gene 203:17-26; Yokoyama-Kobayashi 1997, J Biochem (Tokyo), 122:622-626; Paralkar 1998, J Biol Chem 273:13760-13767). Similar to other TGF-β-related cytokines, GDF-15 is synthesized as an inactive precursor protein, which undergoes disulfide-linked homodimerization. Upon proteolytic cleavage of the N-terminal pro-peptide, GDF-15 is secreted as a ~28 kDa dimeric protein (Bauskin 2000, Embo J 19:2212-2220). Amino acid sequences for GDF-15 are disclosed in WO99/06445, WO00/70051, WO2005/113585, Bottner 1999, Gene 237: 105-111, Bootcov loc. cit, Tan loc. cit., Baek 2001, Mol Pharmacol 59: 901-908, Hromas loc cit, Paralkar loc cit, Morrish 1996, Placenta 17:431-441 or Yokoyama-Kobayashi loc cit.. A preferred GDF-15 assay in the context of the present invention is the assay as described by Wollert et al. in Clinical Chemistry 53, No 2, 2007, p. 284-291.

Determining the amount of sFlt-1, or a cardiac troponin, preferably troponin T, of GDF-15 or of PlGF or any other peptide or polypeptide or protein referred to in this specification relates to measuring the amount or concentration, preferably semi-quantitatively or quantitatively. The terms polypeptide and protein are used interchangeable throughout this application. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of the peptide or polypeptide based on a signal which is obtained from the peptide or polypeptide itself and the intensity of which directly correlates with the number of molecules of the peptide present in the sample. Such a signal – sometimes referred to herein as intensity signal -may be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of the peptide or polypeptide. Indirect measuring includes measuring of a signal obtained from a secondary component (i.e. a component not being the peptide or polypeptide itself) or a biological read out system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products.

In accordance with the present invention, determining the amount of a peptide or polypeptide can be achieved by all known means for determining the amount of a peptide in a sample. Said means comprise immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass- spectrometers, NMR- analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on ElecsysTM analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-HitachiTM analyzers), and latex agglutination assays (available for example on Roche-HitachiTM analyzers).

Preferably, determining the amount of a peptide or polypeptide comprises the steps of (a) contacting a cell capable of eliciting a cellular response the intensity of which is indicative of the amount of the peptide or polypeptide with the said peptide or polypeptide for an adequate period of time, (b) measuring the cellular response. For measuring cellular responses, the sample or processed sample is, preferably, added to a cell culture and an

internal or external cellular response is measured. The cellular response may include the measurable expression of a reporter gene or the secretion of a substance, e.g. a peptide, polypeptide, or a small molecule. The expression or substance shall generate an intensity signal which correlates to the amount of the peptide or polypeptide.

Also preferably, determining the amount of a peptide or polypeptide comprises the step of measuring a specific intensity signal obtainable from the peptide or polypeptide in the sample. As described above, such a signal may be the signal intensity observed at an m/z variable specific for the peptide or polypeptide observed in mass spectra or a NMR spectrum specific for the peptide or polypeptide.

Determining the amount of a peptide or polypeptide may, preferably, comprise the steps of (a) contacting the peptide with a specific ligand, (b) (optionally) removing non-bound ligand, (c) measuring the amount of bound ligand. The bound ligand will generate an intensity signal. Binding according to the present invention includes both covalent and non-covalent binding. A ligand according to the present invention can be any compound, e.g., a peptide, polypeptide, nucleic acid, or small molecule, binding to the peptide or polypeptide described herein. Preferred ligands include antibodies, nucleic acids, peptides or polypeptides such as receptors or binding partners for the peptide or polypeptide and fragments thereof comprising the binding domains for the peptides, and aptamers, e.g. nucleic acid or peptide aptamers. Methods to prepare such ligands are well-known in the art. For example, identification and production of suitable antibodies or aptamers is also offered by commercial suppliers. The person skilled in the art is familiar with methods to develop derivatives of such ligands with higher affinity or specificity. For example, random mutations can be introduced into the nucleic acids, peptides or polypeptides. These derivatives can then be tested for binding according to screening procedures known in the art, e.g. phage display. Antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)2 fragments that are capable of binding antigen or hapten. The present invention also includes single chain antibodies and humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Preferably, the ligand or agent binds specifically to the peptide or polypeptide. Specific binding according to the

present invention means that the ligand or agent should not bind substantially to ("cross-react" with) another peptide, polypeptide or substance present in the sample to be analyzed. Preferably, the specifically bound peptide or polypeptide should be bound with at least 3 times higher, more preferably at least 10 times higher and even more preferably at least 50 times higher affinity than any other relevant peptide or polypeptide. Non-specific binding may be tolerable, if it can still be distinguished and measured unequivocally, e.g. according to its size on a Western Blot, or by its relatively higher abundance in the sample. Binding of the ligand can be measured by any method known in the art. Preferably, said method is semi-quantitative or quantitative. Suitable methods are described in the following.

First, binding of a ligand may be measured directly, e.g. by NMR or surface plasmon resonance.

Second, if the ligand also serves as a substrate of an enzymatic activity of the peptide or polypeptide of interest, an enzymatic reaction product may be measured (e.g. the amount of a protease can be measured by measuring the amount of cleaved substrate, e.g. on a Western Blot). Alternatively, the ligand may exhibit enzymatic properties itself and the "ligand/peptide or polypeptide" complex or the ligand which was bound by the peptide or polypeptide, respectively, may be contacted with a suitable substrate allowing detection by the generation of an intensity signal. For measurement of enzymatic reaction products, preferably the amount of substrate is saturating. The substrate may also be labeled with a detectable label prior to the reaction. Preferably, the sample is contacted with the substrate for an adequate period of time. An adequate period of time refers to the time necessary for a detectable, preferably measurable, amount of product to be produced. Instead of measuring the amount of product, the time necessary for appearance of a given (e.g. detectable) amount of product can be measured.

Third, the ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher

order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand or substrate may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT-BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star™ (Amersham Biosciences), ECF™ (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemoluminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include 35S, 125I, 32P, 33P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latex-enhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labeling or other detection methods as described above.

The amount of a peptide or polypeptide may be, also preferably, determined as follows: (a) contacting a solid support comprising a ligand for the peptide or polypeptide as specified above with a sample comprising the peptide or polypeptide and (b) measuring the amount peptide or polypeptide which is bound to the support. The ligand, preferably chosen from the group consisting of nucleic acids, peptides, polypeptides, antibodies and aptamers, is preferably present on a solid support in immobilized form. Materials for manufacturing solid supports are well known in the art and include, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The ligand or agent may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Suitable methods for fixing/immobilizing said ligand are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like. It is also contemplated to use "suspension arrays" as arrays according to the present invention (Nolan 2002, Trends Biotechnol. 20(1):9-12). In such suspension arrays, the carrier, e.g. a microbead or microsphere, is present in suspension. The array consists of different microbeads or microspheres, possibly labeled, carrying different ligands. Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups, are generally known (US 5,744,305).

Preferably, the amount of a cardiac troponin, sFlt-1, GDF-15 and PlGF and, as the case may be, the amounts of other peptides measured in the context of the present invention are determined in a blood sample, e.g., a serum or plasma sample, obtained from a subject as defined in the present invention. Preferably, such a determination is done by ELISA. Such a determination of sFlt-1 by ELISA can be done, e.g., by using the ELECSYS sFlt-1 test by Roche Diagnostics, Mannheim, Germany. The amount of troponin T can be determined by the COBAS assay, Roche Diagnostics Mannheim, Germany. PGF can be determined with the Quantikine human PlGF immunoassay (Catalogue # DPG00) produced by R&D systems, Minneapolis, USA). GDF-15 can be determined with a polyclonal, GDF-15 affinity chromatography-purified, goat anti-human GDF-15 IgG antibody from R&D Systems (AF957).

The term "amount" as used herein encompasses the absolute amount (e.g., of sFlt-1, a cardiac troponin, GDF-15 or PlGF), the relative amount or concentration (e.g., of sFlt-1, a cardiac troponin, GDF-15 or PlGF) as well as any value or parameter which correlates thereto. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said peptides by direct measurements, e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., expression amounts determined from biological read out systems in response to the peptides or intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

The term "comparing" as used herein encompasses comparing the amount of the peptide, polypeptide, protein comprised by the sample to be analyzed with an amount of a reference source specified elsewhere in this description. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount is compared to an absolute reference amount while a concentration is compared to a reference concentration or an intensity signal obtained from a test sample is compared to the same type of intensity signal of a reference sample. The comparison referred to in step (b) of the method of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount may be compared to values corresponding to references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the amount(s) determined in step a) to reference amount(s), it is possible to diagnose ischemia, reversible cardiac dysfunction and/or non reversible cardiac injury in said subject. It is to be understood that amounts of sFlt-1 or a cardiac troponin as determined in step (a) of the methods of the presents invention are compared in step (b) to reference amounts for sFlt-1 or a cardiac troponin as specified elsewhere in this application.

In general, for determining the respective amounts/amounts or amount ratios allowing to establish the desired result in accordance with the respective embodiment of the present invention, ("threshold", "reference amount"), the amount/amount(s) or amount ratios of the respective peptide or peptides are determined in appropriate subject or patient groups. The term "reference amount" with respect to a cardiac troponin refers, preferably, to an amount

of the cardiac troponin representing the upper level of normal found in a patient without permanent myocardial damage due to physical training. The upper limit of normal is, preferably, defined by the 50th percentile, 55th percentile, 60th percentile, 6^{5th} percentile, 70th percentile, 75th percentile, 80th percentile, 85th percentile, 90th percentile, 90th percentile, 95th percentile or 99th percentile. With respect to a cardiac troponin the term "reference amount", preferably, refers to the amount of a cardiac troponin that allows the differentiation between a patient with and without permanent myocardial damage. With respect to sFlt-1 the term "reference amount" refers, preferably, to an amount of the sFlt-1 representing the upper level of normal found in a subject whose physical training regimen was insufficient to induce angiogenesis. The term "reference amount", preferably, refers to the amount of sFlt-1 that allows the differentiation between a subject whose physical training regimen was successful in inducing angiogenesis and a subject whose physical training regimen was not successful in this respect.

According to the diagnosis to be established, the subject group may, for example, comprise only individuals with successful physical training regimens, or may comprise individuals whose physical training regimen caused permanent myocardial damage, or may comprise only individuals whose physical training regimens failed to induce angiogenesis. The results which are obtained are collected and analyzed by statistical methods known to the person skilled in the art. The obtained threshold values are then established in accordance with the desired probability of successful or unsuccessful physical training and linked to the particular threshold value. For example, it may be useful to choose the median value, the 60th, 70th, 80th, 90th, 95th or even the 99th percentile of the healthy and/or non-healthy patient collective, in order to establish the threshold value(s), reference value(s) or amount ratios.

Consequently, threshold amounts for a cardiac troponin can be derived by determining the amount of the cardiac troponin in individuals who did not experience permanent myocardial damage due to physical training. A reference amount for sFlt-1 is, preferably, derived from a group of subjects without myocardial damage due to the physical training regimen and with or without induction of angiogenesis.

A reference value of a diagnostic marker can be established, and the amount of the marker in a subject sample can simply be compared to the reference value. The sensitivity and specificity of a diagnostic and/or prognostic test depends on more than just the analytical "quality" of the test-they also depend on the definition of what constitutes an abnormal

result. In practice, Receiver Operating Characteristic curves, or "ROC" curves, are typically calculated by plotting the value of a variable versus its relative frequency in "normal" and "disease" populations. For any particular marker of the invention, a distribution of marker amounts for subjects with and without a disease will likely overlap. Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap indicates where the test cannot distinguish normal from disease. A threshold is selected, above which (or below which, depending on how a marker changes with the disease) the test is considered to be abnormal and below which the test is considered to be normal. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification of a condition. ROC curves can be used even when test results don't necessarily give an accurate number. As long as one can rank results, one can create an ROC curve. For example, results of a test on "disease" samples might be ranked according to degree (say l=low, 2=normal, and 3=high). This ranking can be correlated to results in the "normal" population, and a ROC curve created. These methods are well known in the art. See, e.g., Hanley et al, Radiology 143: 29-36 (1982).

In certain embodiments, markers and/or marker panels are selected to exhibit at least about 70% sensitivity, more preferably at least about 80% sensitivity, even more preferably at least about 85% sensitivity, still more preferably at least about 90% sensitivity, and most preferably at least about 95% sensitivity, combined with at least about 70% specificity, more preferably at least about 80% specificity, even more preferably at least about 85% specificity, still more preferably at least about 90% specificity, and most preferably at least about 95% specificity. In particularly preferred embodiments, both the sensitivity and specificity are at least about 75%, more preferably at least about 80%, even more preferably at least about 85%, still more preferably at least about 90%, and most preferably at least about 95%. The term "about" in this context refers to +/- 5% of a given measurement.

In other embodiments, a positive likelihood ratio, negative likelihood ratio, odds ratio, or hazard ratio is used as a measure of a test's ability to predict risk or diagnose a disease. In the case of a positive likelihood ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In the case of a negative likelihood ratio, a value of 1 indicates that a negative result is equally likely among

subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a negative result is more likely in the test group; and a value less than 1 indicates that a negative result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a positive or negative likelihood ratio of at least about 1.5 or more or about 0.67 or less, more preferably at least about 2 or more or about 0.5 or less, still more preferably at least about 5 or more or about 0.2 or less, even more preferably at least about 10 or more or about 0.1 or less, and most preferably at least about 20 or more or about 0.05 or less. The term "about" in this context refers to +/- 5% of a given measurement.

In the case of an odds ratio, a value of 1 indicates that a positive result is equally likely among subjects in both the "diseased" and "control" groups; a value greater than 1 indicates that a positive result is more likely in the diseased group; and a value less than 1 indicates that a positive result is more likely in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit an odds ratio of at least about 2 or more or about 0.5 or less, more preferably at least about 3 or more or about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less. The term "about" in this context refers to +/- 5% of a given measurement.

In the case of a hazard ratio, a value of 1 indicates that the relative risk of an endpoint (e.g., death) is equal in both the "diseased" and "control" groups; a value greater than 1 indicates that the risk is greater in the diseased group; and a value less than 1 indicates that the risk is greater in the control group. In certain preferred embodiments, markers and/or marker panels are preferably selected to exhibit a hazard ratio of at least about 1.1 or more or about 0.91 or less, more preferably at least about 1.25 or more or about 0.8 or less, still more preferably at least about 1.5 or more or about 0.67 or less, even more preferably at least about 2 or more or about 0.5 or less, and most preferably at least about 2.5 or more or about 0.4 or less. The term "about" in this context refers to +/- 5% of a given measurement.

Increased amounts of sFlt-1 as compared to the reference amount indicate an ischemic condition. In muscle-oriented training said ischemia occurs, preferably, in the skeletal muscles. In endurance training ischemia occurs, preferably, in the myocardium. Ischemia is, in principle, a desired effect of physical training, because transient ischemia stimulates angiogenesis in the affected muscles.

Whether angiogenesis is actually induced is, preferably, indicated by PlGF. Increased amounts of PlGF indicate ongoing angiogenesis. In endurance training, PlGF indicates, preferably, angiogenesis in the skeletal muscles. Said angiogenesis may be due to the improving vascularization of existing muscle fibers or due to the vascularization of growing muscle fibers. In endurance training, increased amounts of PlGF indicate, preferably, angiogenesis in the myocardium. Said angiogenesis in the myocardium, preferably, leads to the formation of collaterals to occluded coronary arteries, thus reducing the risk subject's risk of suffering from acute coronary syndromes.

Increased amounts of GDF-15 indicate, preferably, increasing protection against reperfusion injury. In muscle-oriented training GDF-15, preferably, indicates said effect in the skeletal muscles. In endurance training GDF-15, preferably, indicates increased resistance of the myocardium against reperfusion injury.

Cardiac troponins indicate myocardial damage. A moderate increase of a cardiac troponin during physical training is inevitable and does not indicate permanent myocardial damage. However, high increases of a cardiac troponin, preferably, indicate that the extent and/or intensity of physical training causes excessive ischemia and, thus, permanent myocardial damage.

According to the method of the present invention the reference amount is, preferably, based on the amount of the respective marker determined in a sample that was taken before the onset of physical training. Since repeated physical training induces (desired) adaptations of the subject's anatomy (muscle diameter, vascularization of the muscle) and physiology (resistance to reperfusion injury, level of stress required for the induction of angiogenesis), the reference value is, preferably, determined in regular intervals in the context of a physical training regimen. Preferably, said interval is about 1 week, about 2 weeks, about 4 weeks or about 8 weeks. The sample for the determination of the reference amount is, preferably, taken at least about 2 days after the last training session. If it is taken earlier, the amounts of the markers may still be influenced by the aftereffects of the training session. More preferably, the sample for the determination of the reference amount is taken immediately before the start of a training session in order to reflect the current physical condition of the subject.

The increase of the amount of one of the markers of the present invention with respect to the reference amount is, preferably, statistically significant. Whether an increase is

statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student´s t-test, Mann-Whitney test etc.. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the treatment shall be effective for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

More preferably, an increase of:

a) sFlt-1 by at least about 50%, at least about 75%, at least about 100%, at least about 150% or at least about 200%, more preferably of at least about 100%, is indicative for ischemia; and
b) GDF-15 by at least about 20%, at least about 30%, at least about 40%, at least about 60% or at least about 80%, more preferably at least about 40%, is indicative for increasing protection against reperfusion injury;
c) PlGF by at least about 4%, at least about 6%, at least about 8%, at least about 10%, at least about 12% or at least about 15%, more preferably of at least about 8%, is indicative for angiogenesis; and
d) a cardiac troponin by at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 80% or at least about 120%, more preferably of at least about 50%, is considered a high increase and, thus indicative for irreversible myocardial damage.

The previously known methods for the assessing the effects of physical training measure directly (heart rate, VO_{2max}) or indirectly (lactate) the effort of a subject during physical training (see above). However, the effort is not an aim by itself but rather a means for improving the subject's physical capabilities. Thus, the determination of a subject's effort provides - at best - indirect information about the effectiveness of the physical training regimen in question. In contrast to this, the method of the present invention allows the direct monitoring of the desired physiological adaptations caused by physical training. Each of the four biomarkers elucidates a distinctive physiological effect of physical training. Thus, the training regimen can be adapted in order to maximize the desired effect. For athletes in general this means that a training regimen can be chosen that is sufficiently demanding while avoiding overstress. For subjects at increased risk of acute cardiovascular events this means that they can safely practice the sport of their choice without the risk of

precipitating acute cardiovascular events. Since physical training is an important part of cardiovascular rehabilitation programs, the application of the method of the present invention has the potential to improve the effect of cardiovascular rehabilitation significantly, thus reducing morbidity and mortality from acute cardiovascular events.

Furthermore, the present invention relates to a method for determining whether a patient suffered from a myocardial infarction comprising the steps of

a) determining the amount of a cardiac troponin or a variant thereof in a sample of a subject;
b) comparing the amount of the cardiac troponin or the variant thereof determined in step a) to a reference amount;

whereby it is decided whether said patient suffered from a myocardial infarction.

Furthermore, the present invention relates to a method for determining whether a patient suffers from heart failure comprising the steps of

a) determining the amount of a natriuretic peptide or a variant thereof in a sample of a subject;
b) comparing the amount of the natriuretic peptide or the variant thereof determined in step a) to a reference amount;

whereby it is decided whether said patient suffers from heart failure.

Moreover, the present invention relates to a device for assessing the effects of a physical training regimen in a subject comprising

a) an analyzing unit for determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject;
b) an evaluation unit for comparing the amounts determined in step a) to reference amounts.

The term "device" as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to practice the method of the present invention. Preferred means for determining the amounts of the markers of the

present invention, and means for carrying out the comparison are disclosed above in connection with the method of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where an analysis unit for automatically determining the amount of the gene products of the present invention is applied, the data obtained by said automatically operating analysis unit can be processed by, e.g., a computer as evaluation unit in order to obtain the desired results. Preferably, the means are comprised by a single device in such a case.

Said device, preferably, includes an analyzing unit for the measurement of the amount of a cardiac troponin or a variant thereof and the amount of sFlt-1 or a variant thereof in an applied sample and an evaluation unit for processing the resulting data. Preferably, the evaluation unit comprises a database with the stored reference amounts and a computer program code which when tangibly embedded on a computer carries out the comparison of the determined amounts and the reference amounts stored in the database. More preferably, the evaluation unit comprises a further computer program code which allocates the result of the comparison to a risk prediction. In such a case, it is, also preferably, envisaged that the evaluation unit comprises a further database wherein the reference amounts are allocated to the risks.

Alternatively, where means such as test stripes are used for determining the amount of the cardiac troponin or the variant thereof and the amount of sFlt-1 or the variant thereof, the evaluation unit may comprise control stripes or tables allocating the determined amount to a reference amount. The test stripes are, preferably, coupled to ligands which specifically bind to the cardiac troponin and sFlt-1. The strip or device, preferably, comprises means for detection of the binding of said cardiac troponin and sFlt-1 to said ligands. Preferred means for detection are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the analysis unit and the evaluation unit are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual. The analysis unit and the evaluation unit may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however, processed,

i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the gene product, Plasmon surface resonance devices, NMR spectrometers, mass-spectrometers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

Preferably, the analyzing unit of the device of the present invention further comprises means for determining the amount of PlGF or a variant thereof and/or GDF-15 or a variant thereof in a sample of the patient and the evaluation unit further comprises means for comparing the measured amount of PlGF or a variant thereof and/or GDF-15 or a variant thereof with a suitable reference amount.

Furthermore, the present invention relates to a kit for assessing the effects of a physical training regimen in a subject comprising

a) an analyzing agent for determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject;
b) an evaluation unit for comparing the amounts determined in step a) to reference amounts.

The term "kit" as used herein refers to a collection of the aforementioned components of which may or may not be packaged together. The components of the kit may be comprised by separate vials (i.e. as a kit of separate parts) or provided in a single vial. Moreover, it is to be understood that the kit of the present invention is to be used for practising the methods referred to herein above. It is, preferably, envisaged that all components are provided in a ready-to-use manner for practising the methods referred to above. Further, the kit preferably contains instructions for carrying out the said methods. The instructions can be provided by a user's manual in paper- or electronic form. For example, the manual may comprise instructions for interpreting the results obtained when carrying out the aforementioned methods using the kit of the present invention. The kit shall comprise an analyzing agent. This agent is capable of specifically recognizing the peptides of the present invention in a sample of the subject. Moreover, the said agent shall upon binding to the peptides of the present invention, preferably, be capable of generating a detectable signal, the intensity of which correlates to the amount of the peptides of the present invention present in the sample. Dependent on the type of signal which is generated, methods for detection of the signal can be applied which are well known in the art.

Analyzing agents which are preferably used for the kit of the present invention include antibodies or aptamers. The analyzing agent may be present on a test stripe as described elsewhere herein. The amounts of the peptides of the present invention thus detected can then be further evaluated in the evaluation unit. Preferred evaluation units to e used for the kit of the present invention include those referred to elsewhere herein.

Preferably, the analyzing unit of the kit of the present invention further comprises means for determining the amount of PlGF or a variant thereof and/or GDF-15 or a variant thereof in a sample of the patient and the evaluation unit further comprises means for comparing the measured amount of PlGF or a variant thereof and/or GDF-15 or a variant thereof with a suitable reference amount.

The present invention also relates to the use of a kit or device for determining the amount of sFlt-1 or a variant thereof and a cardiac troponin or a variant thereof and optionally PlGF and/or GDF-15 or variants thereof in a sample of a subject, comprising means for determining the amount of sFlt-1 or the variant thereof and the cardiac troponin or the variant thereof and optionally PlGF and/or GDF-15 or variants thereof and/or means for comparing the amount of sFlt-1 or the variant thereof and the cardiac troponin or the variant thereof and optionally PlGF and/or GDF-15 or variants thereof to at least one reference amount for: assessing the effects of a physical training regimen in a subject.

The present invention also relates to the use of: an antibody against a cardiac troponin or a variant thereof and an antibody against sFlt-1 or a variant thereof and optionally GDF-15 or a variant thereof and/or PlGF or a variant thereof for: assessing the effects of a physical training regimen in a subject.

The present invention also relates to the use of: an antibody against a cardiac troponin or a variant thereof and/or an antibody against sFlt-1 or a variant thereof and/or of means for determining the amount of a cardiac troponin or a variant thereof and/or sFlt-1 or a variant thereof, and/or of means for comparing the amount of the cardiac troponin or the variant thereof and/or of the PlGF or the variant thereof to at least one reference amount for: assessing the effects of a physical training regimen in a subject.

Moreover the present invention relates to means for determining the amounts of the aforementioned markers and/or to means for comparing the amounts of said markers to at

least one reference amount for the manufacture of a diagnostic composition for: assessing the effects of a physical training regimen in a subject.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

The following examples are merely intended to illustrate the present invention. They shall not limit the scope of the claims in any way.

Examples

Example 1: Analytical methods

sFlt-1 was determined with a sFlt-1 immunoassay to be used with the Elecsys and COBAS analyzers from Roche Diagnostics, Mannheim, Germany. The assay is based on the sandwich principle and comprises two monoclonal sFlt-1 specific antibodies. The first of these is biotinylated and the second one is labeled with a Tris(2,2'-bipyridyl)ruthenium(TT)-complex. In a first incubation step both antibodies are incubated with the human serum sample. A sandwich complex comprising sFlt-1 and the two different antibodies is formed. In a next incubation step streptavidin-coated beads are added to this complex. The beads bind to the sandwich complexes. The reaction mixture is then aspirated into a measuring cell where the beads are magnetically captured on the surface of an electrode. The application of a voltage then induces a chemiluminescent emission from the ruthenium complex which is measured by a photomultiplier. The amount of light is dependent on the amount of sandwich complexes on the electrode. The test is capable of measuring sFlt-1-concentrations from 10 to 85000 pg/ml.

Troponin T was measured with an immunoassay (hs troponin T) by Roche diagnostics to be used with the above described systems. The test is capable of measuring troponin T-concentrations from 3 to 10000 pg/ml.

GDF-15 was determined with an ELECSYS prototype assay by Roche Diagnostics based on the same principle as set forth above.

PlGF was determined with the Quantikine PlGF immunoassay from R&D Systems Inc. Minneapolis, USA. This assay uses a first anti-PlGF antibody which is immobilized in a polystyrene microplate. This antibody captures the PlGF comprises by the sample. A second anti-PlGF antibody, conjugated to horseradish peroxidase is then added. The amount of PlGF in the sample is determined by measuring the amount of the colored reaction product formed by the horseradish peroxidase from hydrogen peroxide and tetramethylbenzidine.

Example 2: Physical training in healthy subjects

A total of 30 subjects were included in the study, individuals were below the age of 35 years to exclude cardiovascular and other relevant disorders. All individuals underwent a physical examination. The determined parameters included: blood pressure at rest, electrocardiogram at rest as well as a color doppler echocardiography and a stress ECG on a cycle ergometer with the determination of the individual anaerobic threshold and continuous gas exchange measurements to determine the cardiac output.

Among the 30 subjects included in the study 11 were male endurance athletes with an athlete's heart, defined as a volume of at least 13 ml/kg bodyweight as determined by echocardiography, 10 were male athletes with a heart volume below 13 ml/kg bodyweight and 9 were healthy untrained male subjects. They had to exercise on a cycle ergometer at three different intensities in randomized order and a time interval of a least one week between the trials. After the initial stepwise exercise on a cycle ergometer to determine the individual anaerobic threshold the participants had to perform three constant trials on the cycle ergometer for 60 min at an intensity of 70%, 90% and 110% of the individual anaerobic threshold respectively. Venous blood samples were taken before exercise, 30 min after the beginning of exercise, immediately after exercise and 1 hour, 3 hours and approximately 24 hours after exercise.

Table 1: Markers at different times during and after exercise at 90 % of the individual anaerobic threshold

	Before exercise	**During Exercise**	**After Exercise**	**1 hour**	**3 hours**	**24 hours**
sFlt-1 [pg/ml][1]	69 (61-77)	116 (98-129)	145 (117-156)	87 (78-101)	86 (79-91)	64 (59-69)
GDF-15 [pg/ml] [1]	378 (354-419)	415 (369-476)	449 (418-498)	568 (513-698)	539 (473-593)	379 (339-415)
PlGF [pg/ml] [1]	12.9 (11.0-14.9)	13.1 (11.4-15.2)	14.1 (12.1-16.9)	12.7 (10.8-15.3)	9.8 (8.4-12.0)	13.1 (11.8-16.7)
Troponin T [pg/ml] [1]	3.4 (2.2-5.0)	3.5 (2.9-5.0)	4.3 (3.7-6.0)	5.9 (4.7-7.6)	14.4 (8.5-22.8)	4.45 (3.4-6.4)

[1]Values are given as median (25th percentile; 75th percentile)

Table 1 illustrates that sFlt-1, GDF 15, PlGF and Troponin T increase after exercise. sFlt-1 increases can be seen as early as 30 minutes after exercise and peaks at the end of exercise. GDF 15 also starts to increase 30 min after exercise, however, peaks at 1 h after exercise. PlGF increases only slightly after exercise with peaks at the end of exercise. The data obtained are consistent with the induction of exercise induced ischemia, reperfusion injury and temporary induction of angiogenesis. As can also be derived from Table 1, sensitive troponin T increased approximately fourfold to reach its peak at 3 h after exercise indicating exercise induced cardiac damage (necrosis).

Table 2: Comparison of peak levels of the different markers at different levels of exercise

	sFlt-1 [pg/ml][1]	GDF-15 [pg/ml][2]	PlGF [pg/ml][3]	Troponin T [pg/ml][4]
70% anaerobic threshold[5]	92 (80-101)	422 (365-489)	13.1 (10.6-15.2)	5.0 (4.1-7.0)
90% anaerobic threshold[5]	145 (117-156)	568 (512-698)	14.2 (12.1-17.0)	14.4 (8.5-22.8)
110% anaerobic threshold[5]	143 (112-158)	465 (396-536)	14.7 (12.1-17.0)	10.6 (6.6-19.2)

[1]determined immediately after exercise

[2]determined 1 hour after exercise

[3]determined immediately after exercise

[4]determined 3 hours after exercise

[5]Values are given as median (25th percentile; 75th percentile)

As can be seen from the Table 2, sFlt-1 and GDF 15 increases from the 70% to the 90% threshold level but not further, the same is true for troponin T. PlGF increases were only slightly. However, they seemed to be dose dependent. As expected lactate concentrations were 1.2, 2.15 and 8.3 mmol/l for 70%, 90% and 110% threshold levels respectively immediately after exercise. Normal values, i.e. in patients below the anaerobic threshold, range from 0.5 mmol/l to 2.2 mmol/l.

sFlt-1 increase occurred in trained and untrained subjects. sFlt-1 levels tended to increase more in athletes than in untrained subjects. Similar findings were obtained for GDF-15, PlGF and also Troponin T. This indicates that ischemia, angiogenesis and evidence of reperfusion injury as evidenced by GDF-15 increase occurred both in untrained apparently healthy individuals and in trained athletes. This is also the case for cardiac damage as indicated by an increase in Troponin T levels. As the level of exercise required to reach a certain anaerobic threshold is higher in athletes than in untrained individuals, higher levels of the respective markers in athletes are likely to be explained by this observation.

Example 3: Physical training in patients with coronary artery disease

14 patients were included into the study. They were 63 years ± 9 years old. All of them had established stable coronary artery disease, 3 patients had one vessel disease, 3 had two vessel disease and 8 of them had three vessel disease. None of the patients had unstable angina or suffered from dyspnea (The patients had NYHA class 1 heart failure at maximum). All patients had undergone stress ECG and stress echocardiography.

The patients had to walk slowly (approximately 4 km/h) for 30 min or they were allowed to walk at their allowed upper heart rate (brisk walking) for 30 min. Venous blood samples were taken before exercise, immediately after exercise and 1hour and 3 hours after exercise (Scharhag J.et al., 2007, Clin Res Cardiol 96: 218–226).

Table 3A: Markers at different times during and after slow walking					
	Before exercise	**After Exercise**	**1 hour**	**3 hours**	**24 hours**
sFlt-1 [pg/ml][1]	63 (58-64)	77 (75-86)	77 (68-82)	68 (65-76)	65 (62-73)
GDF-15 [pg/ml] [1]	554 (521-776)	668 (528-748)	718 (610-787)	678 (564-798)	642 (501-790)
PlGF [pg/ml] [1]	20.6 (16.8-21.0)	21.9 (18.4-23.5)	19.1 (17.5-21.8)	17.8 (16.2-19.8)	19.0 (16.3-23.3)
Troponin T [pg/ml] [1]	8.4 (6.2-12.7)	9.3 (8.1-14.3)	9.7 (8.3-13.0)	8.9 (7.7-11.9)	9.3 (8.0-12.3)

[1]Values are given as median (25th percentile; 75th percentile)

Table 3B: Markers at different times during and after brisk walking					
	Before exercise	**After Exercise**	**1 hour**	**3 hours**	**24 hours**
sFlt-1 [pg/ml][1]	63 (61-69)	92 (86-104)	75 (70-82)	71 (67-79)	63 (61-69)
GDF-15 [pg/ml] [1]	593 (499-701)	687 (553-735)	797 (623-844)	787 (575-843)	586 (526-757)
PlGF [pg/ml] [1]	19.8 (17.0-21.5)	21.4 (18.7-23.2)	19.6 (17.4-21.6)	17.3 (16.4-19.2)	19.3 (18.0-23.0)
Troponin T [pg/ml] [1]	9.1 (6.9-12.1)	9.9 (7.0-15.2)	9.5 (7.4-14.8)	10.6 (7.1-14.9)	8.6 (7.3-12.4)

[1]Values are given as median (25th percentile; 75th percentile)

The study in patients with coronary artery disease revealed that these patients differed from healthy subjects in that they had higher levels of PlGF, sensitive Troponin T and GDF-15 but not of sFlt-1 at baseline. This is consistent with the underlying diagnosis of chronic artery disease and a stable condition of this disease. After exercise sFlt-1 und GDF-15 increased depending on the level of exercise indicating ischemia and evidence of reperfusion injury. In addition to this, there was evidence of induction of angiogenesis as

indicated by slight increases in PlGF. Significant increases in troponin T were not noted after slow and brisk walking indicating that the extent and intensity of exercise did not induce significant cardiac necrosis. The patients were also devoid of chest pain.

Induction of ischemia as evidenced by increase of sFlt-1 and increase of GDF-15 for protection of reperfusion injury as well as induction of angiogenesis are wanted effects as they induce muscle growth and angiogenesis in the heart to bypass stenoses. Moreover, this protects the body from apoptosis/necrosis in case of a (possibly prolonged) reduction of blood and oxygen supply.

Example 4: General conclusion

Thus the method supplied offers new training methods for healthy and diseased (cardiovascular disease) individuals on an individual basis and allows also to determine the level of exercise that is harmful to the heart. The method can be used in the context of established methods including lactate determination, ECG, echocardiography and others.

Claims

1. A method for assessing the effects of a physical training regimen in a subject comprising the steps of

    a. Determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject;
    b. Comparing the amounts determined in step a) to reference amounts; and
    c. Assessing the effects of the physical training regimen in the subject based on the comparison in step b).

2. The method of claim 1, wherein

    i. an increased amount of sFlt-1 indicates that the intensity ischemic preconditioning is sufficient; and
    ii. an increased amount of the cardiac troponin indicates that the intensity of cardiac preconditioning causes cardiac damage.

3. The method of claim 1 or 2, wherein the amount of PlGF is additionally determined in step a) and wherein an increased amount of PlGF as compared to a reference amount indicates successful adaptation of the myocardium.

4. The method of any of claims 1 to 3, wherein the amount of GDF-15 is additionally determined in step a) and an increased amount of GDF-15 indicates successful adaptation to reperfusion events.

5. The method of any of claims 1 to 4, wherein the cardiac troponin is troponin T.

6. The method of any of claims 1 to 5, wherein the subject is a patient in a cardiac rehabilitation program.

7. The method of any of claims 1 to 5, wherein the subject is a patient who is at increased risk of suffering from acute cardiovascular events.

8. The method of any of claims 1 to 6, wherein the subject is an athlete undergoing endurance training.

9. Use of an antibody binding a cardiac troponin or a variant thereof and of an antibody binding sFlt-1 or a variant thereof for assessing the effects of a physical training regimen in a subject.

10. A device for assessing the effects of a physical training regimen in a subject comprising

    a. an analyzing unit for determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject;
    b. an evaluation unit for comparing the amounts determined in step a) to reference amounts.

11. A kit for assessing the effects of a physical training regimen in a subject comprising

    a. an analyzing agent for determining the amount of sFlt-1 or a variant thereof and of a cardiac troponin or a variant thereof in a sample of a subject;
    b. an evaluation unit for comparing the amounts determined in step a) to reference amounts.

12. The device of claim 10 or the kit of claim 11, further comprising an analyzing unit/agent for determining the amount of PlGF in as sample and an evaluation unit comprising means for comparing the measured amount of PlGF to a reference amount.

13. The device or kit of any of claims 10 to 12, further comprising an analyzing unit/agent for determining the amount of GDF-15 in a sample and an evaluation unit comprising means for comparing the measured amount of GDF-15 to a reference amount.

14. Use of the kit or device according to any of claims 10 to 13 for assessing the effects of a physical training regimen in a subject.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/070535

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 C07K14/71 G01N33/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 090 891 A1 (HOFFMANN LA ROCHE [CH]; ROCHE DIAGNOSTICS GMBH [DE]) 19 August 2009 (2009-08-19)	10-12
Y	page 7, paragraph 35	14

X	F. Grünhage et al.: "IDENTIFICATION OF AN AETIOLOGY-INDEPENDENT PANEL OF SERUM FIBROSIS MARKERS IN PATIENTS STAGED BY TRANSIENT ELASTOGRAPHY", , 15 April 2010 (2010-04-15), XP002613352, Retrieved from the Internet: URL:http://www.kenes.com/easl2010/posters/Abstract288.htm [retrieved on 2010-12-08]	10-13
Y	abstract	14

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2012	16/01/2012

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BAILEY AMELIA PURSER ET AL: "Exercise increases soluble vascular endothelial growth factor receptor-1 (sFlt-1) in circulation of healthy volunteers", MEDICAL SCIENCE MONITOR, vol. 12, no. 2, 1 February 2006 (2006-02-01), pages CR45-CR50, XP008130163, MEDISCIENCE PUBLIKACJE NAUKOWE, WARZAW, PL ISSN: 1234-1010 page CR48, right-hand column, last paragraph - page CR49, right-hand column, last paragraph; figures 1,3	1-9,14
Y	MIDDLETON N ET AL: "Cardiac Troponin T Release Is Stimulated by Endurance Exercise in Healthy Humans", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 52, no. 22, 25 November 2008 (2008-11-25), pages 1813-1814, XP025677232, ELSEVIER, NEW YORK, NY, US ISSN: 0735-1097, DOI: 10.1016/J.JACC.2008.03.069 [retrieved on 2008-11-18] the whole document	1-9,14
Y	WESTERMEYER ET AL: "Elevation of Troponin I in Athletes: A Case Report in a Marathon Runner", JOURNAL OF EMERGENCY MEDICINE, PERGAMON PRESS, NEW YORK, NY, GB, vol. 34, no. 2, 1 February 2008 (2008-02-01), pages 175-178, XP022487237, ISSN: 0736-4679, DOI: DOI:10.1016/J.JEMERMED.2007.03.044 page 178, left-hand column, paragraph 1	1-9,14
Y	URHAUSEN A ET AL: "Clinical significance of increased cardiac troponins T and I in participants of ultra-endurance events", AMERICAN JOURNAL OF CARDIOLOGY, CAHNERS PUBLISHING CO., NEWTON, MA, US, vol. 94, no. 5, 1 September 2004 (2004-09-01), pages 696-698, XP027193405, ISSN: 0002-9149 [retrieved on 2004-09-01] the whole document page 698, left-hand column, last paragraph	1-9,14

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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MINGELS ALMA M A ET AL: "Cardiac troponin T elevations, using highly sensitive assay, in recreational running depend on running distance.", CLINICAL RESEARCH IN CARDIOLOGY : OFFICIAL JOURNAL OF THE GERMAN CARDIAC SOCIETY, vol. 99, no. 6, June 2010 (2010-06), pages 385-391, XP002613320, ISSN: 1861-0692 page 389, right-hand column, last paragraph - page 390, left-hand column, paragraph first	1-9,14
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