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Measurement of Arterial Wall Thickness as a Surrogate Marker for Atherosclerosis

Eric de Groot, MD, PhD; G. Kees Hovingh, MD; Albert Wiegman, MD; Patrick Duriez, PhD; Andries J. Smit, MD, PhD; Jean-Charles Fruchart, PhD; John J.P. Kastelein, MD, PhD

Abstract—Large observational studies and atherosclerosis regression trials of lipid-modifying pharmacotherapy have established that intima-media thickness of the carotid and femoral arteries, as measured noninvasively by B-mode ultrasound, is a valid surrogate marker for the progression of atherosclerotic disease. To exploit fully the potential of ultrasound imaging in atherosclerosis research, standardized and strictly implemented imaging protocols should be used in both observational studies and applied clinical research. This article describes such a protocol developed at the Academic Medical Center of the University of Amsterdam, the Netherlands. Results are presented from a study that estimated atherosclerosis progression from childhood into old age by measuring intima-media thickness in subjects with familial hypercholesterolemia compared with healthy controls. (*Circulation*. 2004;109[suppl III]:III-33–III-38.)

Key Words: B-mode ultrasound ■ familial hypercholesterolemia ■ intima-media thickness ■ surrogate markers

Atherosclerosis is a generalized disease of the arterial wall, which may progress or regress depending on a plethora of factors.^{1–6} This dynamic process is characterized by arterial wall remodeling that may go unnoticed for a lifetime, but may also present as acute vascular disease and become clinically manifest.² Because atherosclerosis progresses over decades, epidemiological studies and intervention trials with clinical end points require long-term follow-up, participation of large populations, or both.^{3–6} These requirements have to be met to provide data from which valid conclusions about the determinants of disease or the efficacy of a therapeutic intervention can be drawn.⁷ As a consequence, such studies consume precious time and financial resources.⁸ To overcome these challenges, surrogate markers became the focus of intense attention.⁸ Such markers might be used to investigate determinants of atherosclerosis at an early stage of the process and can, subsequently, assess modifiers of atherosclerotic disease progression, such as lifestyle and pharmacological interventions.

Boissel and coworkers have proposed criteria for the validity of surrogate markers as a substitute for clinical end points.⁹ These investigators stipulated 3 conditions for the determination of validity. First, a surrogate marker should be more sensitive and more readily available (sensitivity and availability) than the clinical end point. Also, the surrogate marker should be easy to evaluate (convenient), preferably by noninvasive means. Second, the causal relationship between the surrogate marker and the clinical end point (proximity)

should be established on the basis of epidemiological, pathophysiological, and clinical studies. It is a prerequisite that patients with and without vascular disease exhibit differences in surrogate marker measurements (specificity). Third, in intervention studies, anticipated clinical benefits (assessment of benefit) should be deducible from the observed changes in the surrogate marker. The latter argument implies that it is not just cost and time that favors the development of surrogate markers. Validated surrogate markers enable the assessment of promising new drugs in a relatively short period of time at relatively low cost compared with clinical outcome measures and thus obviate the need to await the outcome of trials driven by clinical events.¹⁰ Moreover, the strength of a surrogate marker is enhanced by the fact that it may yield pathophysiological information at an early stage of the disease process. Surrogate markers, therefore, have an inherent value of their own.⁹

Early surrogate markers originated from techniques available for the clinical assessment of patients with vascular disease, such as angiography and Doppler ultrasound. These techniques have significant clinical relevance, but they do not provide useful information on the early stages of arterial wall thickening before lesion formation. Doppler ultrasound can identify a stenosis only when there is a 40% to 50% reduction of the lumen area,¹¹ and angiography can visualize luminal changes only in the very late stages of the disease process. Furthermore, both techniques are inadequate in light of the Glagov effect of initial arterial wall remodeling in the course of atherosclerosis progression.^{12,13}

From the Department of Vascular Medicine (E.d.G., G.K.H., A.W., J.J.P.K.), Academic Medical Center, University of Amsterdam, the Netherlands; the Département de Recherche sur les Lipoprotéines et l'Athérosclérose (P.D., J.-C.F.), Institut Pasteur de Lille, Inserm U545 et Faculté de Pharmacie, Université du Droit et de la Santé de Lille, Lille, France; and the Department of Internal Medicine (A.J.S.), University Hospital, Groningen, the Netherlands.

Correspondence to Prof. Dr J.J.P. Kastelein, Academic Medical Center, Dept. of Vascular Medicine, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. E-mail e.vandongen@amc.uva.nl

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In contrast, B-mode ultrasound imaging technology has evolved to such an extent that the walls of superficial arteries can be imaged noninvasively, in real time and at high resolution. Unlike angiography, or "luminology," ultrasound imaging can visualize the arterial wall itself in every stage of atherosclerosis, from "normal" to complete arterial occlusion. Arterial wall thickness can therefore be measured as a continuous variable from childhood into old age, in patients as well as in healthy controls.¹⁴

Studies evaluating the origin of the lumen-intima and the media-adventitia ultrasound interfaces in relation to carotid and femoral far wall arterial histology have demonstrated that the distance between these interfaces reflects the intima-media complex. Consequently, this distance is referred to as intima-media thickness (IMT).^{15,16} Because B-mode ultrasound is noninvasive, these IMT measurements can be used in observational studies in healthy populations as well as to assess medication efficacy in atherosclerosis regression trials.¹⁶ This article presents a short overview of these observational studies^{17–23} and intervention studies,^{26–30} as well as the B-mode ultrasound imaging protocol as it is presently validated and standardized at the Academic Medical Center in Amsterdam. The need for standardization of IMT measurements in imaging trials is illustrated with recent data from patients with familial hypercholesterolemia (FH) and unaffected (healthy) controls.

Observational Studies and Intervention Trials Using IMT Measurements

Observational Studies

Two large studies that used B-mode IMT measurements to investigate the determinants of atherosclerotic disease in a general population are the Rotterdam Study^{17–19} and the Atherosclerosis Risk in Communities (ARIC) Study.^{20–23} The Rotterdam Study is a single-center, prospective follow-up study of a cohort of 8000 persons >55 years of age living in a suburb of Rotterdam.^{17–19} The objective of this study was to identify the determinants of atherosclerosis progression in the carotid arterial wall. These ultrasound studies provided solid evidence that IMT measurements may indeed be used as an indicator of generalized atherosclerosis.¹⁷ Study results documented associations between carotid IMT and stroke, angina pectoris, myocardial infarction, intermittent claudication, and essential hypertension,^{18,19} as supported by other studies.^{24,25} In the ARIC study^{20–23} in 15 800 American adults, high-resolution B-mode ultrasound was able to assess all stages of atherosclerosis. In ARIC, the procedure showed a high level of reproducibility, was inexpensive, and was established as a noninvasive independent predictor of coronary artery disease. Specifically, in the ARIC study it was observed that a 0.2 mm thicker carotid IMT was associated with a 28% increase in relative risk for stroke and a 33% increase in relative risk for myocardial infarction.

Clinical Trials

B-mode IMT measurements are also used to evaluate the efficacy of lipid-lowering and blood pressure-lowering drugs. The 4-year Cholesterol Lowering Atherosclerosis Study (CLAS)²⁶ assessed the effects of colestipol-niacin therapy in

men who had previously undergone coronary bypass surgery and showed statistically significant ($P<0.0001$) treatment effects after 2 and 4 years of therapy. The Asymptomatic Carotid Artery Progression Study (ACAPS)²⁷ was a 3-year trial investigating the effects of lovastatin 20 to 40 mg per day in asymptomatic men and women between 40 and 79 years of age with early carotid atherosclerosis. Compared with placebo, lovastatin modified the combined IMT of 12 carotid arterial wall segments ($P<0.001$). The Kuopio Atherosclerosis Prevention Study (KAPS)²⁸ investigated the 3-year efficacy of pravastatin in men with hypercholesterolemia aged between 44 and 65 years. The primary outcome measure (combined IMT of 4 carotid arterial wall segments) showed near significance ($P=0.06$), and a highly significant ($P=0.002$) effect was observed on combined (right and left) common carotid IMT. In the 2-year Regression Growth Evaluation Statin Study (REGRESS),^{29,30} pravastatin 40 mg was assessed in men with angiographically proven coronary artery disease and a total cholesterol between 4 and 8 mmol/L (155 and 310 mg/dL). The efficacy of pravastatin was evaluated by coronary angiography²⁹ and by B-mode ultrasound of the peripheral arteries.³⁰ Interestingly, the ultrasound component of REGRESS showed highly significant ($P<0.0001$) improvement in a sample size of only 255 patients. By contrast, improvement at this level of significance was not seen in any of the coronary angiographic parameters of 885 patients of the REGRESS cohort. These findings underline the usefulness of noninvasive ultrasound as a research tool in intervention trials, a field that was dominated until recently by measurements of the coronary artery lumen.

In the 2-year Atorvastatin versus Simvastatin on Atherosclerosis Progression (ASAP) trial,³¹ the effects of atorvastatin 80 mg/d and simvastatin 40 mg/d were investigated in 325 patients with FH. Aggressive cholesterol lowering had a greater effect on atherosclerosis progression than did conventional statin treatment. Specifically, the study showed an actual decrease in carotid IMT in the more aggressively treated group which was on 80 mg of atorvastatin ($\pm 51\%$ decrease in low-density lipoprotein cholesterol [LDL-C]), whereas less aggressive treatment ($\pm 41\%$ LDL-C lowering) showed only inhibition of progression. The outcome of the recent 1-year Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) study³² in 161 patients with cardiovascular disease is in line with the ASAP findings. The effects of atorvastatin 80 mg/d and pravastatin 40 mg/d on carotid IMT were compared to investigate whether lowering LDL-C below the National Cholesterol Education Program (NCEP) criterion for secondary prevention (100 mg/dL; 2.59 mmol/L) would further reduce the burden of atherosclerotic disease. Atorvastatin lowered LDL-C by 49%, to 76 (± 23) mg/dL, and pravastatin by 27%, to 110 (± 30) mg/dL. In the pravastatin group, IMT stabilized (increase of 0.025 ± 0.017 mm), whereas in the atorvastatin group the IMT decreased (by 0.034 ± 0.021 mm), showing atherosclerosis regression ($P=0.03$). Possibly, lowering LDL-C even beyond present guidelines has a favorable effect on arterial walls and consequently on the risk of future cardiovascular disease.

IMT Is a Validated End Point for Atherosclerotic Vascular Disease

Considering all of the above, IMT measurements acquired by means of B-mode ultrasound imaging of carotid and femoral arterial walls meet all validity criteria of a surrogate marker. Moreover, B-mode ultrasound can provide data on peripheral arterial wall thickness in all stages of atherosclerosis. Prospective epidemiological studies have shown that an increase in IMT due to cardiovascular risk factors is associated with an increase in relative risk for myocardial infarction and stroke, and that a decrease in IMT due to drug treatment is associated with a decrease in the incidence of vascular events. In particular, in the REGRESS trial,^{29,30} it was observed that statins have a favorable impact on the coronary lumen, IMT of carotid and femoral arteries, and clinical cardiovascular events. Therefore, IMT has now been accepted as a validated marker for the process of atherosclerosis.

Standardized B-Mode Ultrasound Imaging and Image Analysis Protocol

To exploit fully the potential of ultrasound imaging in atherosclerosis research, standardized and strictly implemented imaging protocols should be used both in observational studies and in applied clinical research. The ultrasound protocol summarized below has been successfully applied in such studies and in atherosclerosis regression trials. The dynamic changes in the process of atherosclerosis, whether due to genetic or environmental factors or resulting from lipid-lowering drug treatment, were entered into a model. In our view, standardization of image acquisition provides complementary observational and trial data. Hundreds of subjects may be included in observational studies and lipid-lowering intervention trials, often in multicenter settings. Consequently, large numbers of images are generated. This necessitates a protocol that has been designed for efficiency of image acquisition and image analysis.

In our B-mode ultrasound protocol, the common carotid, the carotid bulb, the internal carotid, the common femoral, and the superficial femoral arterial far wall segments were scanned bilaterally. ACUSON 128XP™ ultrasound instruments (Acuson, Mountain View, Calif) equipped with 5 to 10 MHz linear array broadband L7 transducers and Extended Frequency (EF) software were used. A standard view of 2×2 centimeters was imaged. Images were saved as 4:1 compressed JPEG files.^{33,34} These image files are approximately 150k each, a size that can be easily transferred over the Internet (Figure 1). Image acquisition and image analyses have been digitized according to DICOM standards.³⁵ This approach allows for high-quality ultrasound image capture,³⁶ and makes it possible to control measurements from image acquisition to manuscript writing and to anticipate and conform with regulatory guidelines for drug trials using image-based measurements as end points. In clinical research, image analyses are performed in the controlled environment of an ultrasound core laboratory, where validated hardware and software should be used. The associated off-line measurement is illustrated in Figure 2. Output of the image analysis consists of a text file with demographic, quality assessment, and measurement data as well as an

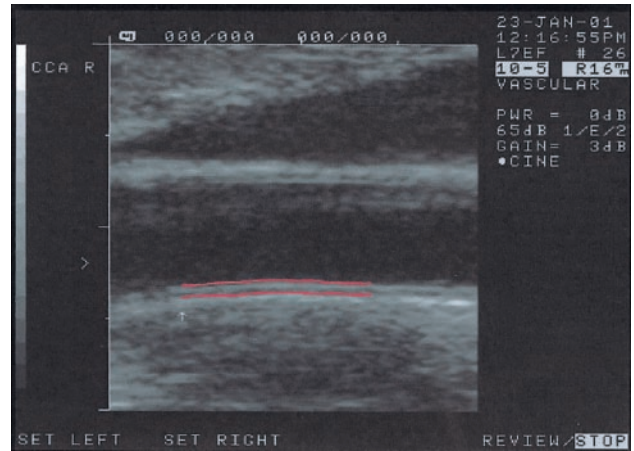


Figure 1. A 2×2-cm B-mode ultrasound digital still image of the common carotid artery and its adjacent structures as depicted by a 5 to 10 MHz linear array transducer. The white triangle on top is the sternocleidomastoid muscle; the black triangle over the common carotid is the jugular vein. The common carotid near and far arterial walls are clearly shown. The common carotid segment is defined as the arterial wall proximal (to the right) of the carotid dilatation (small white arrow). The lumen-intima and the media-adventitia interfaces are illustrated in red.

associated 7k-sized control JPEG image file that shows how the measurement was done (Figure 3). Standardized image acquisition is a key issue in arterial wall imaging studies. The use of digital imaging techniques and the Internet allows for a more user-friendly study environment. This can be illustrated by data comparing patient populations known to be at high risk for cardiovascular disease and healthy people at low risk.

Standardized IMT Measurements in Persons at High Cardiovascular Risk

The ultrasound protocol described above was used to investigate carotid and femoral IMT in patients with FH and

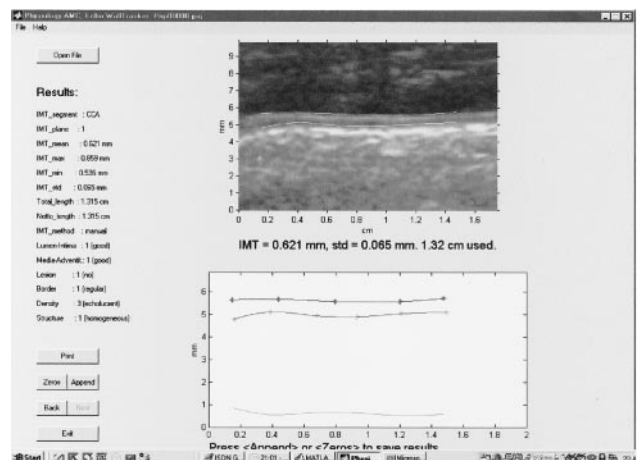


Figure 2. The ultrasound digital still images are imported as 4:1 compressed JPEG files into a dedicated software program (etrack). In the image, a region of interest is identified by the reader. The IMT of this particular arterial far wall is 0.621 ± 0.065 mm. (Courtesy Dr W.J. Stok, MD, and Dr J.M. Karemaker, MD, PhD, Departments of Vascular Medicine and Physiology, Academic Medical Center, Amsterdam, the Netherlands.)

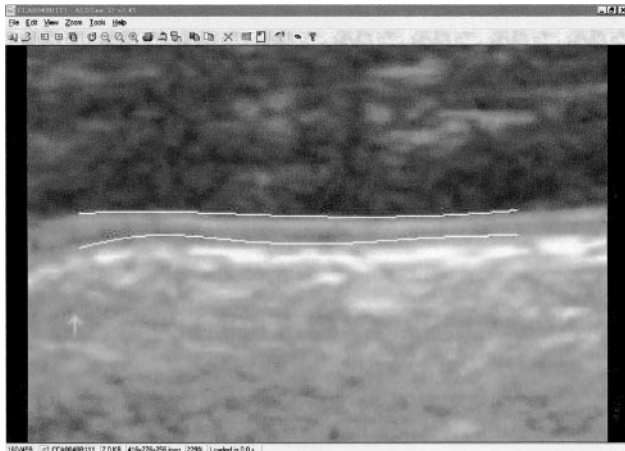


Figure 3. The software program provides IMT data as well as an associated control JPEG image of 7K with every arterial wall thickness measurement. These images allow for sonography and image analysis quality control and quality assurance issues.

unaffected siblings. FH is a common autosomal dominant disorder of lipoprotein metabolism, affecting approximately 1 in 400 persons in the Netherlands.³⁷ Patients have elevated levels of LDL-C due to mutations in the LDL-receptor gene. As a result of their excessively high LDL-C levels, FH patients are at very high risk for premature cardiovascular disease.³⁸ At the present time, cardiovascular disease risk due to FH can be reduced only by lipid-lowering agents. This need for lipid-lowering therapy and the age that treatment should be started are important questions for parents of a child with FH.

To estimate atherosclerosis progression from childhood into seniority in controls and in FH subjects, we used cross-sectional standardized IMT measurements in 6 unaffected and affected age groups. Because measurements were standardized, we could extrapolate atherosclerosis progression estimates from these cross-sectional data for each of the groups and, consecutively, for the combined groups as a whole. This approach does not obviate the need for longitudinal studies and clinical end point data, but it does circumvent the need to perform lifelong studies to describe vascular

wall changes and substantiates the need for preventive measures in persons at high cardiovascular risk.

The clinical and demographic characteristics of the FH subjects and controls are given in the Table. Carotid and femoral IMT was measured in all subjects and combined to a per-subject average. First, for each data set, characteristics of arterial walls were correlated with age and vascular risk. In children at age 10, mean carotid IMT was similar in FH subjects and controls (both, 0.53 ± 0.03 mm; $P > 0.15$). Then, the progression estimates were extended to age 76 to estimate atherosclerosis progression from childhood into old age. Lowess splines were used for the scatter of each data set (Figure 4, black dotted lines). These splines consequently indicated a similar linear increase in estimated IMT with age in all 6 populations. Second, the data on FH (mean IMT 0.79 ± 0.20 mm; range, 0.45 to 1.53 mm) and unaffected populations (mean IMT 0.63 ± 0.14 mm; range, 0.48 to 1.14 mm) were pooled into 2 data sets. The FH group consisted of 315 subjects (age range, 11 to 67 years; LDL-C 7.2 ± 1.8 mmol/L); the control group consisted of 118 unaffected subjects (age range, 11 to 76 years; LDL-C 3.1 ± 0.8 mmol/L). We then applied an overall linear regression analysis to estimate IMT increase with age between groups and found that IMT increase with age was at least twice as large in FH subjects as in controls (0.009 and 0.004 mm/year, respectively). The mean differential IMT change between FH and controls was 0.005 mm/year ($P < 0.001$, Figure 4).

Discussion

These observations led us to estimate that, on average, a healthy person reaches an IMT of 0.78 mm at the age of 76 years. In FH patients this IMT is already reached at the age of 40 years. Moreover, as can be observed from the scatterplots of Figure 4, linear regression, although statistically correct, is a poor representation of the disruption (and the accompanying symptoms of disease) of arterial walls that emerges once an arterial wall thickness of approximately 0.8 mm is reached. In our opinion, it is precisely that stage of arterial wall disruption that should be prevented by aggressive lipid-lowering therapy. Our analyses indicate rapid athero-

Clinical and Biochemical Characteristics of Unaffected Controls and FH Subjects

Population Samples	Controls			FH Subjects		
	Adolescents	Middle-Aged	Seniors	Adolescents	Young Adults	Older Adults
N	44	26	48	44	23	248
Gender, male/female	22/22	11/15	24/24	23/21	12/11	128/120
Age, y	14.9±2.8	34.9±9.6	67.4±7.6	14.8±2.8	28.5±8.8	48.1±8.8
Total cholesterol, mmol/L	4.2±0.7	6.3±1.8	5.9±1.1	7.8±1.9	7.5±1.8	9.6±1.9
LDL-C, mmol/L	2.5±0.6	2.7±0.8	3.9±1.1	6.1±1.8	5.5±1.7	7.6±1.9
HDL-C, mmol/L	1.4±0.3	1.5±0.4	1.3±0.4	1.2±0.3	1.3±0.4	1.2±0.3
Triglycerides, mmol/L	0.8±0.3	1.3±0.4	1.7±0.24	1.0±0.3	1.6±0.6	1.9±0.9
IMT, mm	0.53±0.03	0.59±0.07	0.77±0.12	0.55±0.05	0.65±0.08	0.86±0.18

Major demographic and lipid characteristics of 3 unaffected groups and 3 groups with familial hypercholesterolemia (FH). IMTs were calculated as the population means of the per-subject combined and averaged carotid and femoral IMT measurements. IMT indicates intima-media thickness; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

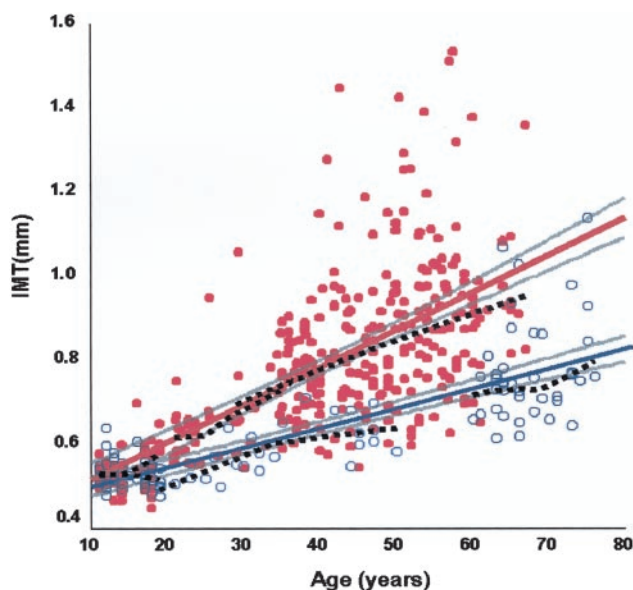


Figure 4. Arterial wall thickness progression estimates in persons heterozygous for familial hypercholesterolemia (FH; LDL-C 7.2 ± 2.0 mmol/L; IMT 0.79 ± 0.20 [range, 0.45 to 1.53] mm; red dots) and healthy controls (LDL-C 3.4 ± 0.8 mmol/L; IMT 0.63 ± 0.14 [0.48 to 1.14] mm; blue dots). For the pooled FH (red line) and the pooled control data (blue line), IMT increase was estimated by linear regression (with respective 95% confidence intervals depicted in gray lines). On average, healthy controls reach an IMT of approximately 0.8 mm at age 80, whereas FH subjects reach this value (and, if untreated, often their first cardiovascular symptoms) around the age of 40 years.

sclerosis progression in FH subjects and are in line with the fact that coronary artery disease will manifest before the age of 50 years in many persons with FH. This IMT graph therefore not only illustrates the opportunity to study effective new drugs in populations such as FH, but also shows the need for primary prevention in young persons with the disorder. The short time available for cardiovascular disease prevention in FH patients emphasizes the need for interventions to modify atherosclerosis progression at a very early time point in life.

IMT measurements can accurately describe the process of arterial wall changes due to atherosclerosis as a continuous in vivo variable. IMT measurements can also provide information on apparently healthy and at-risk populations. In addition, IMT measurements can provide data on the efficacy of novel lipid-modifying medications. It may therefore be concluded that IMT measurements are a validated surrogate end point for atherosclerosis and vascular disease risk. Lastly, if IMT outcome is to be used as an argument in discussions of whether or not to apply preventive measures in presumed at-risk populations, and of whether the results of studies evaluating the therapeutic response to drugs are valid for the general population, the strength of the argument is better supported if the IMT measurements are performed in a standardized environment.

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