Détermination du débit cardiaque par la méthode du Modelflow appliquée à une artère périphérique au repos et à l'exercice modéré

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Détermination du débit cardiaque par la méthode du Modelflow appliquée à une artère périphérique au repos et à l'exercice modéré

[*Measurement of Cardiac Output by the Modelflow method applied on a peripheral artery at rest and at moderate exercise*]

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Intitulée :

DETERMINATION DU DEBIT CARDIAQUE PAR LA METHODE DU MODELFLOW APPLIQUEE A UNE ARTERE PERIPHERIQUE AU REPOS ET A L'EXERCICE MODERE

La Faculté de médecine, sur le préavis de Monsieur Michel Muhlethaler, profeseur ordinaire au Département des Neurosciences Fondamentales et du Docteur Guido Ferretti, privat-docent au Département des Neurosciences Fondamentales, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

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N.B. - La thèse doit porter la déclaration précédente et remplir les conditions énumérées dans les "Informations relatives à la présentation des thèses de doctorat à l'Université de Genève".

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Voici votre deuxième doctorat !

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RESUME

INTRODUCTION

Effectuer un exercice musculaire sous-maximal à l'état stationnaire requiert l'hydrolyse de l'ATP dont la resynthèse est due au métabolisme aérobique, et implique ainsi une consommation d'oxygène ($\dot{V}_{O_2}$), laquelle implique à son tour des réajustements cardio-vasculaires, notamment en ce qui concerne le débit cardiaque ($\dot{Q}$). L'équation de Fick décrit la relation entre $\dot{V}_{O_2}$ et $\dot{Q}$ comme suit : $\dot{V}_{O_2} = \dot{Q} . (C_{aO_2} - C_{\bar{v}O_2})$ où C_{aO_2} et et $C_{\bar{v}O_2}$ sont les contenus en oxygène respectivement du sang artériel et du sang veineux mêlé. Les travaux de Cerretelli et al., (1987), ont montré que la relation entre $\dot{Q}$ et $\dot{V}_{O_2}$ est linéaire et positive (fig.1).

Du point de vue fonctionnel, l'augmentation de $\dot{Q}$ pendant l'exercice est due à l'augmentation de la fréquence cardiaque (HR) et du volume d'éjection systolique (SV). Au total, il y a augmentation de la perfusion musculaire squelettique, concomitante à une diminution des résistances périphériques. L'ensemble de ces modifications produit des effets discordants au niveau de la pression artérielle.

REAJUSTEMENTS CARDIOVASCULAIRES A L'EXERCICE

Modifications de la fréquence cardiaque : L'augmentation de HR se fait par le biais de l'activation du centre de régulation cardio-respiratoire. Deux théories principales sont évoquées pour expliquer cette activation: la théorie de la *commande centrale* qui décrit le recrutement d'unités motrices par des signaux efférents du cortex moteur, et la théorie dite du *réflexe moteur* qui attribue cette activation du centre cardio-respiratoire à des signaux afférents venant des muscles squelettiques en travail.

Toutefois, l'augmentation de HR et de la $\dot{V}_{O_2}$ a également été observée en contexte d'absence de possibilité « anatomique » de feedback neurologique, notamment chez les traumatisés de la moelle épinière et chez les transplantés cardiaques, ce qui suggère l'hypothèse du métaboréflexe selon laquelle la tachycardie pendant l'exercice pourrait aussi

être le résultat de l'influence de métabolites d'origine musculaire, et/ou de libération de la catécholamines d'origine surrénalienne dans le sang.

Modifications du volume d'éjection systolique : La composante la plus significative de SV pendant l'exercice est d'origine mécanique ; elle est due au mécanisme de Frank-Starling, selon lequel l'augmentation du retour veineux provoque un volume télédiastolique plus important, ce qui implique une plus grande distension de la paroi ventriculaire, d'où une plus grande force contractile, et donc une augmentation de SV. Les composantes de cette augmentation du retour veineux pendant l'exercice sont nombreuses. On peut citer le passage de la position debout à la position couchée, *l'effet pompe* des groupes musculaires actifs sur le réseau veineux, et le jeu de pression transdiaphragmatique qui crée un *effet de succion* intermittente sur la veine cave inférieure et le réseau veineux intra-abdominal.
En plus de ces phénomènes mécaniques, des mécanismes neurologiques ont aussi été évoqués pour expliquer l'augmentation de SV, notamment l'inotropisme dû à la stimulation sympathique.

Effets sur la pression artérielle : Toute baisse de la pression artérielle inclut un risque d'hypoperfusion des organes périphériques. Ce risque est levé grâce au baroréflexe qui provoque à court terme une tachycardie et une vasoconstriction réflexes, et donc le maintien d'une pression de perfusion efficace. Le maintien de la pression artérielle à long terme est le fait de mécanismes réno-hormonaux de régulation de la volémie.

La mesure expérimentale de $\dot{Q}$ est une étape-clé pour une meilleure compréhension des réajustements cardiovasculaires à l'exercice. Différentes méthodes de mesure invasives et non-invasives ont été utilisées jusqu'à présent.

METHODES DE MESURE DU DEBIT CARDIAQUE

Méthodes conventionnelles à l'état stationnaire

Méthodes invasives de Fick (directe et indirectes) : Sur la base de l'équation de Fick, on détermine $C_{\bar{v}O_2}$ par cathétérisme de l'artère pulmonaire, et C_{aO_2} par prélèvement sanguin périphérique. A l'état stationnaire, on peut mesurer $\dot{V}_{O_2}$ par circuit-ouvert conventionnel (sac

de Douglas), et calculer $\dot{Q}$. Bien que cette méthode soit considérée comme référence, elle comporte quelques limitations liées non seulement aux situations d'interêt de mesure non stationnaire, mais aussi à son caractère invasif, notamment chez les sujets en bonne santé ; par ailleurs, elle requiert une technicité pour la pose du cathéter. La méthode par dilution de colorant ou par thermodilution sont également dérivées de l'équation de Fick (méthodes de Fick indirectes).

Méthodes non invasives ; principe des méthodes des gaz inertes : La prise pulmonaire de gaz inertes (éthylène, oxyde nitrique, ou acétylène) solubles dans le sang est directement proportionnelle au débit sanguin pulmonaire, lequel est considéré équivalent à $\dot{Q}$ à l'état stationnaire. Différents protocoles de respiration ou re-respiration existent depuis la description de la méthode par Grollman. La méthode la plus répandue est la re-respiration d'acétylène à 0,5 ou 1% dans un mélange gazeux contenant 35 à 45% d'O_2, 5 à 10% d'hélium (gaz insoluble), et du N_2. La mesure des fractions partielles des gaz est faite dans ce cas par spectrométrie de masse, permettant le calcul de $\dot{Q}$. Les limitations de cette méthode sont surtout notées au repos.
Une alternative à la re-respiration est la méthode de la « respiration unique » décrite par Kim et coll. en 1966. Elle est très peu utilisée en raison du choix empirique de la valeur du quotient respiratoire qui doit être fait, alors que la courbe de dissociation du CO_2 n'est pas linéaire.

Techniques récentes : Non invasives, état stationnaire non requis

L'échocardiographie Doppler permet une détermination non invasive battement-par-battement de $\dot{Q}$, donc sans besoin d'être à l'état stationnaire. Toutefois, certains inconvénients limitent l'usage de cette technique en physiologie de l'exercice, notamment le niveau de technicité requis pour la manipulation. Durant l'exercice, il y a deux sources potentielles de biais de la mesure, à savoir : l'angulation de la sonde et le choix du site de mesure dans la crosse aortique ; ces 2 paramètres influencent la valeur de la section du vaisseau qu'on calcule.

Cardiographie d'impédance : Le principe de l'impédance, décrit en 1966 par Kubicek, est basé sur la spécificité de l'impédance tissulaire. Selon la loi de Kirchov, le courant électrique passe par les circuits de forte conductance, un courant alternatif (4 mA, 100 kHz) appliqué sur le tronc considéré statique, permet de déterminer la variation d'impédance de la crosse

aortique, et d'en déduire le débit cardiaque. La principale limitation dans cette technique vient des mouvements induits par l'exercice.

Méthode du Modelflow (analyse du profil de l'onde de pression) : Appelée Méthode du contour de pouls [Stok et al. (1993); Antonutto et al. (1994) Antonutto et al. (1995)] ou Modelflow® [Wesseling et al., 1993], cette technique requiert encore quelques améliorations.

En plus de la mesure de pression faite sur un court trajet artériel, la résistance artérielle doit être connue, pour servir dans le calcul du débit. Cette résistance inclut les propriétés vasculaires (visco-élasticité, géométrie) et celles de la colonne sanguine (inertie, viscosité, présence d'ondes refléchies). Etant donné qu'il s'agit d'un flux pulsé (court segment artériel), le terme d'impédance vasculaire est utilisé en lieu et place de « résistance pulsatile ». Pour des raisons pratiques d'approximation, l'impedance caractéristique qui est la composante de l'impédance vasculaire directement liée aux propriétés visco-élastiques du segment artériel considéré, est plus usitée. Ceci exclut les phénomèmes liés à la colonne de sang.

Selon la méthode du Modelflow®, SV est le résultat de la division de l'aire sous la courbe de l'onde de pression, par l'impédance (Z) de la circulation sanguine. Les caractéristiques de Z (il s'agit ici de l'impédance caractéristique) sont déterminées sur la base d'algorithmes issus des travaux de Langewouters sur une série de mesures post-mortem mentionnant l'âge et le statut cardio-vasculaire. Le modèle-à-3éléments, sur le principe duquel est bâtie la méthode du Modelflow® initialement développée pour la crosse de l'Aorte, est implémenté pour le traitement du profil de l'onde pulsatile périphérique enregistré au bout du doigt. Ceci introduit un risque d'erreur en raison des différences morphologiques existant entre la périphérie (doigt) et le profil de pression centrale (figure 8). Les travaux de Houtman et al. (1999) ont toutefois validé la mesure de $\dot{Q}$ au bout du doigt, en ouvrant une discussion sur la fiabilité de la méthode à l'exercice, du fait des modifications de l'activité sympathique et des résistances périphériques totales.

BUT, METHODES, RESULTATS ET CONCLUSION DE LA THESE :

Le but de ce travail était d'évaluer le Modelflow® comme méthode de mesure de $\dot{Q}$ au repos et à l'exercice modéré, sur la base de deux hypothèses, à savoir : i) Les résultats de Houtman et al. (1999) étaient la conséquence de mesures périphériques. ii) L'inexactitute de mesure par le Modelflow® est le fait d'une erreur systématique, corrigeable par une calibration adéquate.

Les résultats de ces deux études ont été publiés en deux articles originaux, à savoir Azabji et al. (2004), et Tam et al. (2004) repris dans cette thèse **[Azabji Kenfack *et al.*, 2004; Tam *et al.*, 2004]**.

Pour tester la première hypothèse (première étude), après obtention de l'accord du Comité d'éthique local, 7 volontaires sains non fumeurs (âge = 24.0 ± 2.9 ans, poids corporel = 81.2 ± 12.6 kg), informés du protocole expérimental et des risques potentiels, ont été inclus dans l'étude. Nous comparons les valeurs de $\dot{Q}$ obtenues par Modelflow® à partir des profils de l'onde pulsatile enregistrés simultanément au doigt et sur l'artère radiale, au repos et à l'exercice. Les profils pulsatiles périphériques se traduisent par des valeurs de $\dot{Q}$ systématiquement plus élevées que celles obtenues au niveau artériel.

Dans la deuxième étude, 9 volontaires (âge = 24.6 ± 2.96 ans; poids corporel = 74.6 ± 6.90 kg; taille = 180.4 ± 4.03 cm) sont inclus. Nous avons déterminé $\dot{Q}$ par calibration du Modelflow® appliqué aux profils pulsatiles au doigt, en utilisant la technique d'acétylène en circuit-ouvert, au repos et à divers niveaux d'effort à l'état stationnaire. Les facteurs de correction obtenus étaient indépendants du niveau d'effort. L'utilisation du Modelflow® comme méthode fiable pour la mesure de $\dot{Q}$ au repos et à l'exercice est possible seulement après correction par rapport à une méthode indépendante.

GENERAL INTRODUCTION

1 FICK'S EQUATION

The mechanical work of exercise is sustained by the hydrolysis of ATP during muscular contraction. During moderate exercise at the metabolic steady state, ATP resynthesis is supported by aerobic metabolism, which implies an increase in oxygen consumption ($\dot{V}_{O_2}$). The latter requires a functional readjustment of the cardiovascular system, in accordance with the Fick principle:

$$\dot{V}_{O_2} = \dot{Q} . (C_{aO_2} - C_{\bar{v}O_2}) \qquad (1)$$

where $\dot{Q}$ is cardiac output, C_{aO_2} is arterial blood oxygen concentration, and $C_{\bar{v}O_2}$ is mixed venous oxygen concentration. Equation (1) implies that there is a linear positive relationship between $\dot{Q}$ and $\dot{V}_{O_2}$, as illustrated in Figure 1 **[Cerretelli *et al.*, 1987]**:

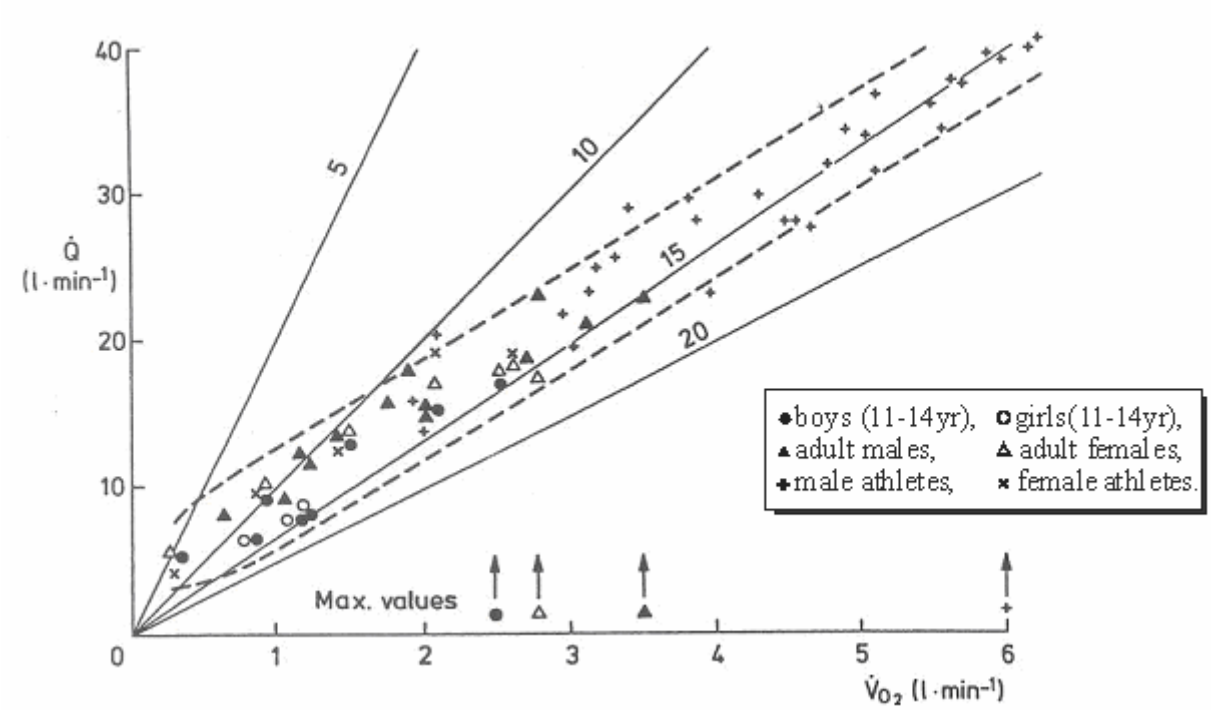

Figure 1. **$\dot{Q}$ as a function of O_2 uptake ($\dot{V}_{O_2}$) for different groups of subjects.** Group $\dot{V}\max_{O_2}$ values are shown by ↑. Continuous straight lines converging on the origin of the axes are isopleths for arterial-mixed venous O_2 difference, expressed in milliliters of O_2 per 100 ml of blood : the corresponding value is indicated above each line**. [Cerretelli & Di Pamprero, 1987]**

$\dot{Q}$ is also equal to the product of heart rate (HR) times the stroke volume of the heart (SV). Thus, the $\dot{Q}$-increase during exercise is the result of an increase of both HR and SV. The increase of the former is of neural origin, that in the latter may have both a neural and a mechanical (Frank-Starling mechanism) origin. Moreover, there is an increase in muscle blood flow, with consequent decrease in peripheral resistance. The ensemble of these changes have discordant effects on arterial blood pressure. These readjustments are briefly overviewed in the next paragraphs.

2 CARDIAC OUTPUT READJUSMENTS

2.1 HEART RATE

The HR increase at exercise onset is mediated by the activation of areas in the brain stem responsible for the cardio-respiratory regulation. This activation may have a dual origin. On one side, a *central command* theory states that it is caused by signals descending from regions of the motor cortex recruiting motor units **[Ebert, 1986]**. On the other side, an exercise motor reflex theory attributes this activation to afferent signals arising from contracting muscles **[Carrington *et al.*, 2001]**. Whichever is the case, the outcome is a modification of the sympathetic and vagal control of the heart activity. Studies with vagal and/or sympathetic blockade have shown that: i) the initial HR answer (in the case of a frequency from 60 to 100 bpm) is almost exclusively due to the reduction in vagal tonicity that intervenes instantaneously at exercise onset **[Fagraeus *et al.*, 1976]**, and: ii) above a frequency of 100 bpm, the HR increase is due to the activation of the sympathetic system, that means, to a more efficient stimulation of the cardiac adrenergic receptors **[Cerretelli, 2002]**. For steady-state aerobic submaximal exercise, HR is a linear function of $\dot{V}_{O_2}$.

However, an increase in HR in response to an increase of $\dot{V}_{O_2}$ occurs even in the absence of intact neural feedback and motor center activity, as for example in humans affected by a spinal cord injury and in heart transplant recipients **[Kjaer *et al.*, 1999; Marconi *et al.*, 2003]**. This suggests that the tachycardia observed during exercise could also be influenced by metabolites arising from the working muscles, or by a release of catecholamines from adrenal glands into blood.

2.2 STROKE VOLUME

2.2.a Mechanical augmentation of SV (position/gravity, muscle pump action)

The most important component of the SV increase during exercise has a mechanical origin, being due to the Frank-Starling mechanism. An increase in venous return leads to a higher telediastolic volume, which implies a greater distension of the ventricle wall. This carries along a greater force of contraction by the myocardium, with consequent increase of SV. At the cellular level, the increase in contraction force is a consequence of the increase in passive tension of the elastic elements in-series of lengthened cardiac myocytes **[Patterson *et al.*, 1914; Starling *et al.*, 1926; Fye, 1983; Zimmer, 2002; Helmes *et al.*, 2003; Levy *et al.*, 2005]**. The same mechanism explains why, for any given HR value, SV is some 20% larger in supine than in upright position **[Margaria *et al.*, 1970]**. Also, the SV increase during a rest-to-exercise transition is larger (some 50 to 70%) in the upright than in the supine position **[Higginbotham *et al.*, 1986]**. At exercise, the increase in venous return is immediate, and is due to the pump action of the active muscle mass on its venous network **[IO *et al.*, 2003]**. Furthermore, during exercise, intrathoracic pressure becomes more negative and the movements of diaphragm intermittently increase the pressure gradient between the thorax and the abdomen, which both also contribute to increase venous return by a "suction" effect of the inferior vena cava on the intra-abdominal veins **[Lloyd, 1983; Takata *et al.*, 1992; Carry *et al.*, 1994; Kitano *et al.*, 1999]**.

2.2.b Implication of neurologic mechanisms

The increase in $\dot{Q}$ upon exercise onset may also have a neural origin, because of the inotropic effect of sympathetic stimulation. Two mechanisms potentially stimulating the sympathetic system at exercise have been proposed: 1) excitation of free nerve endings within the skeletal muscle ("metaboreceptors") by some exercise metabolic end-products (lactic acid, arachidonic acid, bradykinin and potassium), and: 2) mechanical activation of free nerve endings **[Rodman *et al.*, 2003]**. The generic term "ergoreceptor" is often used to name any free nerve ending which would stimulate sympathetic nerves in a muscle at exercise, either by a metabolic or a mechanical stimulation.

2.3 THE BAROREFLEX

Any drop of the systemic blood pressure involves a risk of hypoperfusion in the peripheral organs. Numerous mechanisms are involved in counteracting a pressure drop. Baroreflexes are responsible for a short term response, whereby reflex tachycardia and vasoconstriction maintain an effective blood pressure.

The blood pressure is due to the force which the intravascular blood exerts on the vessel wall. Due to its pumping action, the heart also generates a pressure, in the purpose of creating a flow. From this view, we know that :

$$\dot{Q} = \frac{\Delta P}{R} \qquad (2)$$

where ΔP is the pressure difference between aorta and vena cava and R is the total peripheral resistance.

It is thus important to know how P and R vary. The baroreflex is the fundamental physiological actor of the short-term regulation of the arterial blood pressure. This short-term regulation is mediated by baroreceptors. These are stretch-sensors, placed in the vascular wall. Baroreceptors are located in the arch of the aorta and at the carotid sinus. Action potentials are transmitted to Nucleus Tractus Solitarius, those generated from the former location by the vagus nerve, those from the latter location by the glosso-pharyngean nerve. The integrated response to baroreceptor stimulation is a decrease in HR, by vagal stimulation and sympathetic inhibition. As a result, the work of the heart is reduced, as is arterial blood pressure.

Baroreceptors are stretched when the tension of the vascular wall is increased, so they are sensitive to variations in blood-pressure, even of the order of a few mmHg. The cellular or molecular events during transduction of pressure input into afferent sympathetic nerve signals are far from being understood. Yet, some aspects of this mechanism have been identified **[Imaizumi *et al.*, 1993; Sato *et al.*, 1998; Minson *et al.*, 2000; Kawada *et al.*, 2005]**. Sugimachi et al. **[Sugimachi *et al.*, 1990]** analysed the discharge of baroreceptors exposed to provoked random pressure perturbations. They showed that aortic arch baroreceptors respond primarily to dynamic rather than to static changes in pressure.

In addition there are **low pressure baroreceptors**, located in the right auricle. Functionally similar to pulmonary mechanoreceptors (stimulated at the inspiration), they also send messages to the Nucleus Tractus Solitarius. Their role is to fit the degree of activation of the autonomic nervous system to the venous blood-pressure and to variations of the intrathoracic blood-pressure due to respiration.

We will not comment here on the long-term regulation of arterial blood pressure (controlled essentially by modification of the plasmatic volume by the kidney via hormones: Renin-Angiotensin-Aldosteron-System, Vasopressin, Natriuretic atrial factor), which is outside the scope of this thesis. An interested reader may look into some review papers **[Bader *et al.*, 2001; Vennervald *et al.*, 2004].**

2.4 PERIPHERAL DISTRIBUTION OF BLOOD FLOW

In general, there is an increase in systolic blood pressure during muscular exercise. However, the mean blood pressure increases only moderately. This means that increases in blood flow during exercise are due essentially to the reduction of total peripheral resistance **[Escourrou *et al.*, 1993]**. Such a reduction, clearly established in the active muscular groups, is a consequence of peripheral muscle vasodilatation, and carries along a redistribution of circulating blood to the active muscle mass. This blood flow redistribution, which is under the effect of local metabolic modifications, is also accompanied by vasoconstriction in the visceral vessels **[Osada *et al.*, 1999]**, except the vital organs (i.e the brain).

According to the importance of the muscular groups in activity, vasodilatation can be sufficient to induce an "obliteration" of the baroreflex. But the combination of the increase of HR and the moderate augmentation of the blood pressure involves a displacement of the curve of the baroreflex to the top and right side, so inducing a change in the operational range pressure of the baroreflex (figure 2). This phenomenon is also called "resetting" of the threshold of the baroreflex **[Potts *et al.*, 1993; Papelier *et al.*, 1994; Iellamo *et al.*, 1997; Yamamoto *et al.*, 2004]**.

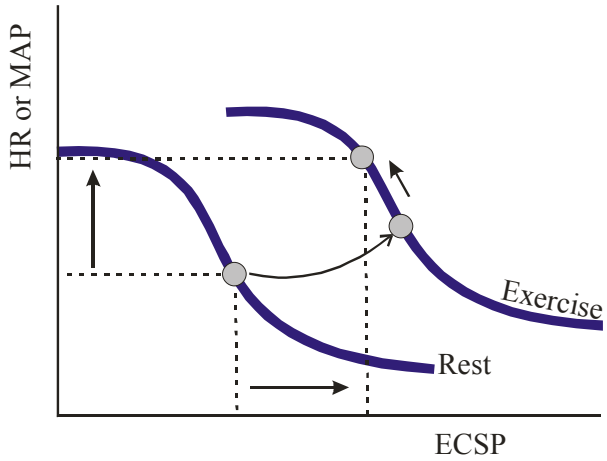

Figure 2. Schematic representation of the carotid baroreflex function curve at rest and its subsequent relocation during exercise (modified from **[Potts *et al.*, 1993]**). MAP, mean arterial pressure; ECSP, Estimated Carotid Sinus Pressure.

MEASUREMENT OF CARDIAC OUTPUT

The measurement of $\dot{Q}$ has been a key issue in our experimental understanding of the cardiovascular responses to exercise. $\dot{Q}$ at rest and during submaximal steady-state exercise has been investigated since long, by a series of invasive and non invasive methods.

1 CONVENTIONAL TECHNIQUES

1.1 STEADY-STATE INVASIVE MEASUREMENTS

1.1.a Direct Fick

The direct Fick method is an application of Fick principle. Thus, it requires simultaneous measurement of the concentration of O_2 in arterial (C_{aO_2}) and mixed venous ($C_{\bar{v}O_2}$) blood, and of the rate of O_2 consumption ($\dot{V}_{O_2}$). At the steady state, $\dot{V}_{O_2}$ can be classically measured by close-circuit spirometry with CO_2 absorption, and by the conventional open-circuit method (Douglas bag method). More recently, methods for breath-by-breath $\dot{V}_{O_2}$ determination were also introduced **[Auchincloss *et al.*, 1966; Capelli *et al.*, 2001]**. $C_{\bar{v}O_2}$ is measured on blood sampled from a catheterized pulmonary artery. C_{aO_2} is measured on blood sampled from a systemic artery (peripherally). This method is generally considered the most direct, reliable and reproducible method for steady-state $\dot{Q}$ measurements. However, it has some drawbacks, limiting its application to highly-controlled hospital conditions. This method in fact requires highly trained personnel to perform manipulation of catheters. Furthermore, it carries along significant risk for numerous potential complications (including damage to carotid and subclavian artery, pneumothorax, dysrhythmias, perforation of chamber of the heart, tamponade, valve damage, pulmonary artery rupture and catheter knotting **[Boyd *et al.*, 1983; Horst *et al.*, 1984]**. These risks are too high for allowing its use on healthy volunteers in exercise physiology laboratories.

The accuracy is largely determined by the investigator's ability to maintain the subject at steady-state. This condition is rarely achieved during incremental exercise up to the maximum. At rest, the margin of error is approximately 5% **[Holmgren *et al.*, 1960]**.

1.1.b Dye-dilution

This is also called "indicator-dilution method". In its basic principle, the method is similar to direct Fick method with the exception that, instead of measuring $\dot{V}_{O2}$, the concentration of a dye is measured. In practice, a known amount of the dye, usually indocyanine green, is injected into the right heart via a the vena cava, by a catheter. A few seconds later, the dye concentration is detected in downstream arterial blood, by withdrawing small samples of blood continuously from an arterial catheter and measuring the dye concentration with a densitometer. The dye concentration gradually increases until it reaches a maximum, and then it begins to decrease until a second rise in concentration occurs as a result of recirculation. Summing the concentration of each sample and dividing by the number of samples will give the average concentration of dye. For a given amount of injected dye, the amount of dye that has been passing through the sampling site per unit of time is directly proportional to the area of the dye-dilution curve and inversely proportional to the flow of blood at the sampling site : the larger the area under the curve, the smaller the $\dot{Q}$ (see Fig. 2).

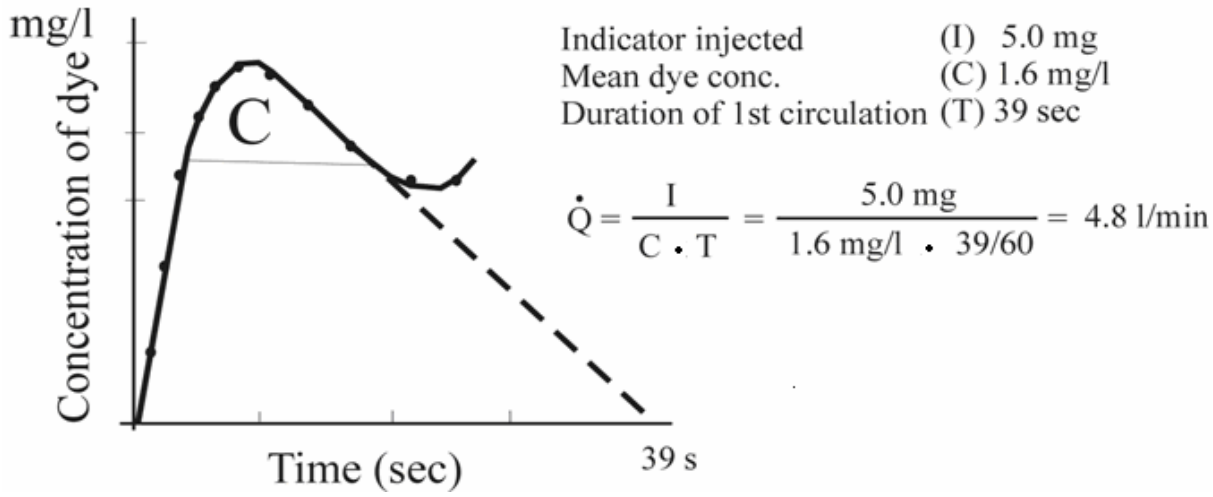

Figure 3. $\dot{Q}$ determination by indicator dilution. Plot of time vs. the logarithm of indicator dye concentration in the aorta after injection of 5mg of indicator (left). The indicator disappearance time without recirculation is obtained by extrapolation. The area of the curve is represented by the product of C x T and the $\dot{Q}$ is the quotient of the amount injected divided by the area of the curve **[Smith *et al.*, 1990]**.

The dye-dilution technique has the same degree of accuracy and reproducibility as the direct Fick method **[Maccanon *et al.*, 1955]**. This technique is easier to use than the direct Fick method, since it does not strictly require cardiac catheterisation. However, it is still too

invasive for a general use in exercise physiology, although many adaptations have been proposed.

1.1.c Thermodilution

This method is a different application of the dye-dilution principle, whereby, instead of dye, a cold fluid (bolus of ice-cold dextrose or saline solution) is injected into the right atrium; blood temperature is then measured by a thermistor near the end of the pulmonary artery. The degree of temperature dilution depends on the flow, and, in a manner similar to dye dilution, a temperature-time curve is inscribed, and the amount of blood cooling is inversely proportional to $\dot{Q}$ **[Smith & Kampine, 1990]**.

This technique has a precious advantage over the dye-dilution method : concerning the marker, recirculation is of little concern, and doesn't produce any change in its baseline, so that numerous measurements can be made. Yet, an unknown amount of the coolant may be lost during the handling of the syringes and in the catheter before it enters the circulation. Physiological variations may also occur in the temperature of the pulmonary blood as a result of phasic heat exchange of air in the lungs.

Nevertheless, the use of this technique as a *gold standard* is doubtful. Several authors reported consistent overestimation in comparison with the dye-dilution and indirect respiratory methods based on CO_2 **[Branthwaite *et al.*, 1968; Espersen *et al.*, 1995]**.

1.2 STEADY-STATE NON INVASIVE MEASUREMENTS

1.2.a Foreign gas measures of cardiac output

The general principle of these techniques relies on the notion that the rate of disappearance from the lungs of inert soluble gases, which can enter or leave the bloodstream freely, is directly proportional to lung blood flow, which is considered equivalent to $\dot{Q}$ at the steady state.

In exercise physiology, two soluble inert gases are commonly used, Nitrous oxyde (N_2O) and Acetylene (C_2H_2), both administered with rebreathing and non-rebreathing procedures. Acetylene has been the choice of many exercise physiologists, because N_2O is thought to be significantly affected by the blood lipid levels **[Simmons *et al.*, 1971]**.

In its first version, Grollman used 4% Ethylene (instead of Acetylene) in a normobaric oxygen mixture balanced with nitrogen. After a measurement of steady-state $\dot{V}_{O_2}$ by a usual method, the subject rebreathed 5 to 6 times in 15 seconds approximately, in order to equilibrate the gas mixture in the lung-bag system. A sample was then taken from the bag in an evacuating tube. After further 12 seconds of rebreathing, a second sample was taken. An analysis of the 2 samples gave the necessary data for calculating the arterio-venous oxygen difference, and then $\dot{Q}$ by means of the following equation:

$$\dot{Q} = \frac{\dot{V}_{O_2}}{123 \bullet \frac{B - 48.3}{760} \bullet \frac{(C_2H_4)_{AV}}{100} \bullet \frac{(O_2)_{II} - (O_2)_{I_{corr}}}{(C_2H_4)_{II} - (C_2H_4)_{I_{corr}}}} \qquad (3)$$

where B is the atmospheric pressure during the experiment ; $(C_2H_4)_{AV}$ is the average concentration of acetylene in the alveoli, during the period of rebreathing ; each $(X)_{I_{corr}}$ is the % of gas X, measured in the first sample in the mixture of lung-bag volume, and corrected for the lung volume ; $(X)_{II}$ is the % of gas X, measured in the second sample in the mixture of lung-bag volume. Solubility of Ethylene: the constant 123 is used since 1 liter of blood (at BTPS conditions) absorbs 123 cm^3 of ethylene.

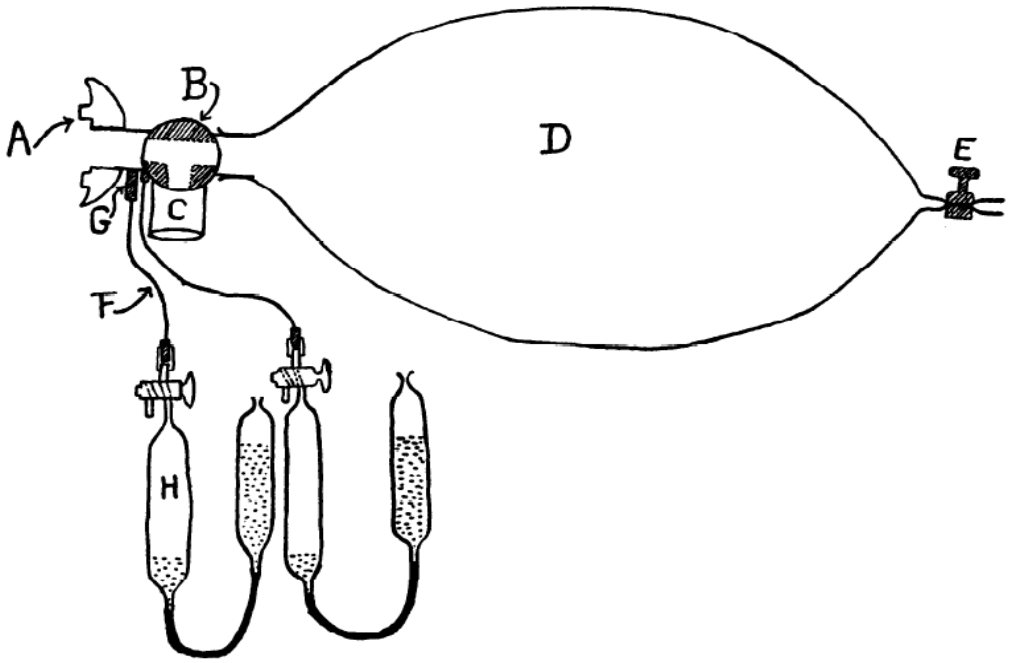

Figure 4. A rubber bag, D, of about 2.5 to 3.0 liters capacity is attached to a large three-way metal tap, B, similar to that used in respiratory work with the Douglas bag. A mouth-piece, A, is placed on the other end of the tap so that the subject can breathe either from the outside air, C, or from the bag, D. Between the mouth-piece and tap two small flexible metallic tubes, F, of 0.5 mm bore are inserted, and the other ends arranged to fit over evacuated mercury sampling tubes, N.

In the most commonly used C_2H_2 technique, the volunteer rebreathes a gas mixture containing 35 to 45% O_2, 0.5 to 1% C_2H_2, 5 to 10% Helium (insoluble gas), and a balance of N_2 **[Triebwasser *et al.*, 1977]**. The insoluble gas is used to assess adequate mixing of the lung-bag rebreathing system and any reduction in the rebreathing volume. Breath-by-breath analysis of the expired gases can be made by a mass spectrometer. The changes observed in the concentration of C_2H_2 after adequate mixing and before recirculation are then used to calculate $\dot{Q}$.

The subject rebreathes the gas mixture at a rate of approximately 1 breath / 1.5 s at rest and 1 breath / s during maximal exercise, for a duration of approximately 18 and 10 s respectively. The equilibration between lungs, dead space and bag usually occurs within the third breath. Therefore, the first 3 breaths are discarded. C_2H_2 mixes in the lung-bag system, then it disappears in the blood in a linear fashion according to $\dot{Q}$ and to the solubility coefficient of the inert gas in blood. After the equilibration point, the rate of decline in the C_2H_2 is directly proportional to lung blood flow, and thus to $\dot{Q}$ **[Hsia, 1998; Warburton *et al.*, 1998]**. If the $\dot{V}_{O_2}$ during the C_2H_2 rebreathing manoeuvre is measured, the formula for the calculation of arteriovenous difference for oxygen content is as follows:

$$C_{aO_2} - C_{\bar{v}O_2} = \frac{(O_2) \bullet ({}_xC_2H_2) \bullet (P_b - P_{H_2O}) \cdot \lambda}{C_2H_2} \qquad (4)$$

where O_2 and C_2H_2 are the amounts of oxygen or acetylene absorbed during the time of the sampling, xC_2H_2 is the average concentration of C_2H_2 during the time of the sampling, P_b is the barometric pressure, P_{H_2O} is the vapour pressure at saturation in the lungs – which at 37 °C is equal to 47 mmHg – and λ is a constant derived from the solubility coefficient of C_2H_2 in blood **[Wagner *et al.*, 1974; Meyer *et al.*, 1980]**.

At rest, foreign gas rebreathing techniques are not accurate as invasive methods, due to the artificial elevation of $(C_{aO_2} - C_{\bar{v}O_2})$ caused by rebreathing, leading to an underestimate of $\dot{Q}$. There is also a significant limitation in using these techniques on subjects with pulmonary abnormalities, for there is inefficient mixing of gases in the lungs **[Jones, 1975]**. With this limitation in mind, foreign gas techniques can be used to give non invasive, simple and valid

determinations of $\dot{Q}$ during submaximal and maximal exercise. This is of key importance to exercise physiologists who are primarily concerned with the changes in $\dot{Q}$ during exercise.

1.2.b Single-breath method

To avoid changes occuring in $(C_{aO_2} - C_{\bar{v}O_2})$ by the rebreathing itself, Kim et al. proposed a single-breath method **[Kim *et al.*, 1966]**. The basic principle is that, during a prolonged breath holding, the rise of arterial and alveolar CO_2 concentrations is non-linear, while O_2 concentration decreases linearly **[Kim *et al.*, 1966; Hlastala *et al.*, 1972]**.

The instantaneous respiratory gas exchange ratio (R_{inst}) is known to be linearly related to the expired partial pressure (P_{CO2}) of CO_2. These are used to estimate mixed venous ($P_{\bar{v}CO_2}$) and arterial (P_{aCO_2}) CO_2 tensions.

In case of normal blood pH, as at rest and during light exercise, it has also been demonstrated that, at an instantaneous respiratory exchange ratio (R) of 0.32, taking into account the Haldane effect, the expired CO_2 tension turns out equal to $P_{\bar{v}CO_2}$, and that P_{aCO_2} is the tension of CO_2 in arterial blood, determined as the average value for the previous 6 breaths **[Kim *et al.*, 1966]**. On this basis, $\dot{Q}$ can be determined as:

$$\dot{Q} = \frac{\dot{V}_{O2} \bullet (R - 0.32)}{S \bullet (P_{\bar{v}CO_2} - P_{aCO_2})} \qquad (5)$$

where S is the slope of the CO_2 dissociation curve. Despite marked differences in the accuracy between rebreathing and single-breath methods, their reproducibility is similar at rest and at moderate exercise **[Kim *et al.*, 1966]**.

The reliability of this method has been questioned. Luft UC et al. **[Luft, 1973]** observed that single-breath estimates of P_{aCO_2} and $P_{\bar{v}CO_2}$ differed from corresponding measured values. According to Inman et al. **[Inman *et al.*, 1985]**, there is consistent underestimation of end-tidal P_{CO_2} values from the single-breath method. In order to account for the Haldane effect, theoretical simulations aiming at a represention of the point of true $P_{\bar{v}CO_2}$ implied an empirical choice of the appropriate R value. Chen et al. **[Chen *et al.*, 1982]** chose a value of R=0.38 as opposed to 0.32 by Kim et al. **[Kim *et al.*, 1966]**, whereas Loeppky **[Loeppky *et al.*, 1983]**, using a mathematical description of the CO_2 dissociation curve to quantitate the *in vivo*

Haldane factor (fH), computed R=0.28, which reduced the difference between the $\dot{Q}$ values obtained with single-breath and rebreathing methods.

Since the CO_2 dissociation curve is not linear, especially during exercise, and the principle of single-breath method approximates the kinetics of P_{aO_2} and P_{aCO_2} by linear functions, the most formal analysis that explains inaccuracy of the method is given by Gronlund **[Gronlund, 1982]**: "*The basic source of errors in the data-reduction procedure seems to be the use of a fitted polynomial to computer derivatives of a non polynomial experimental curve*."

2 NOVEL TECHNIQUES: NONINVASIVE – UNSTEADY STATE

2.1 ECHOCARDIOGRAPHY

When a high frequency wave sound (above $2*10^6$ cycles/sec) is emitted through the heart, the depth and position of the echoes returned from the body can be detected and recorded, and transduced in graphics called "echocardiograms". This is the principle of echocardiography.

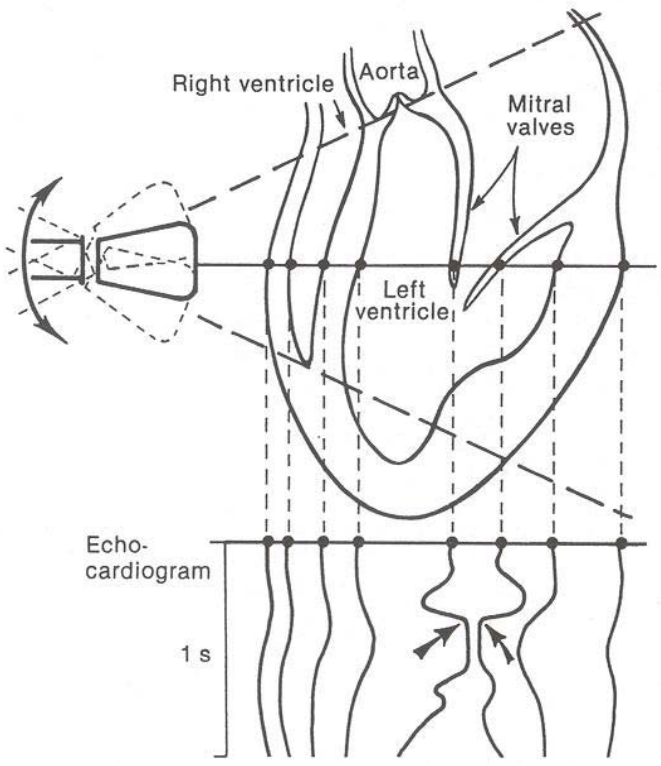

Figure 5. Schematic diagram of the principle of echocardiography. The ultrasonic beam is swept in an arc between apex and base. The transducer acts as both sender and receiver in rapid alternation. The distances and movements of the reflecting surfaces are recorded as a function of time. The closing of the mitral valve at the beginning of systole, for example, can be visualized **[Smith & Kampine, 1990]**.

A refinement of this technique allows the recording of velocity of blood flow within the heart and great vessels. For a stationary measure device, the frequency of sound or light waves reflected by a moving source changes in relation to the speed at which the source is moving. This change in frequency is called "Doppler effect", in honour of Christian Doppler, who first described this phenomenon in 1842. The refined technique is thus called Doppler Echocardiography. The device comprises a transmitter and a sensor of ultrasounds. After emitting an ultrasound at a given frequency, the sounds reflected back from a column of moving blood is recorded by the sensor at a different frequency. The extent of that change in frequency depends on the velocity of blood flow, this velocity can be calculated by the Doppler equation **[Rowland *et al.*, 2002]**:

$$V = \frac{c \bullet (Fr - Ft)}{2 \bullet Ft \bullet \cos\theta} \qquad (6)$$

where V is the true velocity of the blood, c is the speed of sound, Ft is the emitted frequency, Fr is the received frequency, and θ is the angle between the transmitted beam and the direction of blood flow.

The Doppler signal for each heart beat is displayed as a plot of velocity of blood flow over time. The velocity-time integral (VTI) times the cross-sectional area (CSA) through which the blood is passing (aorta cross), gives the SV :

$$SV = VTI \bullet CSA \qquad (7)$$

$\dot{Q}$ is then determined by multiplying SV by HR.

Doppler echocardiography has a series of advantages which makes it a suitable choice for exercise physiologists. It provides a simple and accurate system by which state changes in $\dot{Q}$ during resting and exercise conditions can be monitored noninvasively on a beat-by-beat basis.

However, there are some disadvantages that limit the use of the Doppler method in exercise physiology **[Shaw *et al.*, 1985]**. This method requires trained personnel to operate, with a good knowledge of the anatomy of the heart and aortic blood flow characteristics. Especially during exercise, there are two added potential sources of error: transducer angulation and the site of aortic cross-sectionnal area measurement.

Transducer angulation: As long as the angle between the ultrasound transmitter beam and the aorta is more than 20°, the cosine will be significantly lower than 1, and the inaccuracy and imprecision of the measure of the blood flow velocity may become important. Velocity is

usually underestimated by 6, 13 and 29% at angles of 20, 30 and 45% respectively **[Rowland & Obert, 2002]**. At 90°, the velocity will be recorded as zero (see figure 5).

Site of aortic sectional area measurement: To optimise the accuracy, the measure should be done on the highest velocity sites, in other words, on the smallest CSA. A 5% error in diameter determination causes 10% error in SV calculation since the value for the measured diameter is squared in the determination of area **[Shaw *et al.*, 1985]**. The difficulty to maintain the beam at the exact site during exercise is of capital importance.

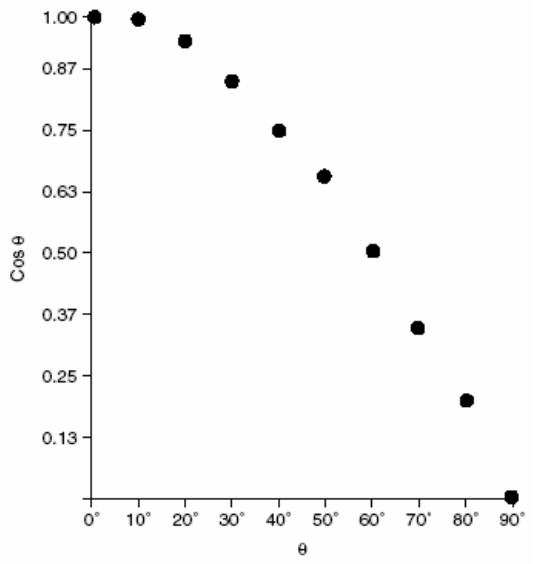

Figure 6. Error in measured aortic velocity produced by changes in angle (θ) between the ultrasound transducer and ascending aortic blood flow. **[Rowland & Obert, 2002]**

2.2 IMPEDANCE CARDIOGRAPHY

Biologic tissues behave electrically like a resistor-capacitor. The electrical bioimpedance technology uses constant electric current stimulation for identification of electrical impedance variations, which are associated with changes in the body. The specific impedance of a given tissue (blood for example) is close to its resistivity. The experimental measures of resistivity, expressed in ohms per centimetre, is 150 for blood, 63 for plasma, 750 for cardiac muscle, 1275 for lungs, and 2500 for fat **[Moshkovitz *et al.*, 2004]**.

This means that the resistivity of blood and plasma is the lowest. Then, according to the Kirchov law, electric current passes through conduits of higher conductance; thus, when an alternate current of 20-to-100 kHz is applied, it is primarily distributed via the extracellular fluid and the blood vessels. Consequently, for each systolic increase in the aortic blood volume, there is a proportional increase in the aortic electrical conductance.

The analysis of thoracic impedance waveform, based on examination of the pulsatile changes in resistance occurring during ventricular systole and diastole, was first introduced in 1966 by Kubicek **[Kubicek *et al.*, 1966].** In this method, a small alternating current (4 mA, 100 kHz) is passed through the chest using 2 sets of electrodes at the base of the neck and at the lower chest **[Kubicek *et al.*, 1966]**. Changes in electrical impedance are measured by 2 other sets of recording electrodes. Pulsatile changes in thoracic aortic blood flow cause changes in baseline thoracic impedance (Zo) measured as ΔZ. Thoracic impedance is known to be linearly related to aortic blood flow and can be expressed by its first derivative (dZ/dt). Changes in transthoracic electrical impedance during systole are representative of SV. $\dot{Q}$ can therefore be calculated from SV and HR **[Fuller, 1992; Raaijmakers *et al.*, 1999; Hartleb *et al.*, 2000; Imhoff *et al.*, 2000]**.

Several formulas have been developed for the electrical bioimpedance estimation of SV. Sramek **[Sramek *et al.*, 1983]** proposed a model of the thorax as a truncated cone, and their formula is as follows:

$$SV = VEPT \bullet LVET \bullet \frac{(dZ/dt)_{min}}{Z_o} \qquad (8)$$

where VEPT is the volume of electrically participating tissue, calculated by the formula: $\frac{L^3}{4.2}$ *(L is the distance between recording electrodes). LVET is the Left Ventricular Ejection Time.*

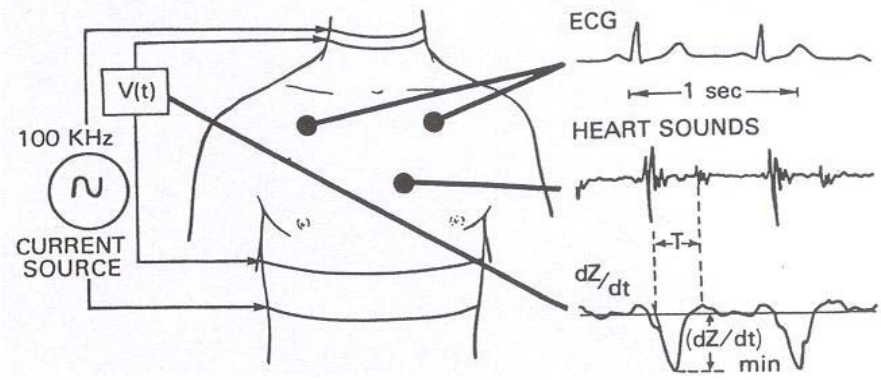

Figure 7. Transthoracic impedance cardiography. **[Smith & Kampine, 1990]**
Left, Rapid sinusoidal current is transmitted through the two outer electrodes and sensed by the inner ones.
Right, the minimum value of the first derivative of the main impedance wave (dZ/dt_{min}) and the ventricular ejection time (T) are used to calculate the stroke volume.

The electrical bioimpedance model meets most of the criteria for use during exercise. It is noninvasive, cost-effective and suitable for continuous monitoring of cardiac function **[Jensen *et al.*, 1995]**. But there are some sources of error to take in account. One is related to

electrode placement: the measurement site of the inner pair of electrodes used in the determination of L has a large effect on the accuracy of impedance cardiography **[Denniston *et al.*, 1976]**. Haematocrit and intrathoracic fluid shifts also influence the accuracy of bioimpedance measurements **[Edmunds *et al.*, 1982]**.

When used to assess cardiac function during exercise, impedance cardiography can be problematic due to movements of the trunk during strenuous exercise conditions **[Miles *et al.*, 1981; Hetherington *et al.*, 1985]**.

2.3 METHODS BASED ON PRESSURE PROFILE ANALYSIS

The above analysis has demonstrated that the beat-by-beat methods of $\dot{Q}$ determination by echo-Doppler or electric impedance present serious drawbacks, especially when they are applied at exercise. Methods based on arterial pulse pressure contour analysis are most promising, but still require refinements. Two methods have been proposed, namely the pulse contour **[Stok *et al.*, 1993; Antonutto *et al.*, 1994; Antonutto *et al.*, 1995]** and the Modelflow® **[Wesseling *et al.*, 1993]** methods.

On a limited arterial traject, it is usually admitted that the resistance to flow include : (i)visco-elastic properties of the vessel wall and the geometry of the blood vessel; (ii) inertia and viscosity of the blood column ; (iii) and the reflection of the flow and pressures waves.

Since in a portion of elastic artery the flow is generally pulsed, this pulsatile resistance, also termed resistance to phasic flow, is usually called "vascular impedance". The expression "hydraulic impedance" is also often used. One component of vascular impedance is termed "characteristic" impedance, wich is directely related to the viscoelastic properties of the blood vessel. Characteristic impedance exclude all events related to the flow (reflected waves, viscosity, density)**[Smith & Kampine, 1990; d'Alché, 1999]** .

2.3.a Principle of the pulse contour method

In this method, SV is obtained as :

$$SV = As \bullet Z^{-1} \qquad (9)$$

Where **As** is the area underneath the systolic pressure profile (in mm of Hg times second), and **Z** (in mm of Hg times second per ml) is the apparent hydraulic impedance of the circulatory system. According to geometric and structural characteristics of the system, Z is considered as an empirical calibration factor, computed by means of published algorithms **[Langewouters**

***et al.*, 1984]** which take into account some of the subject's physical characteristics (age, cardio-vascular status).

2.3.b Principle of Modelflow® :

It is admitted that arteries have 2 functions: (i) since they are tubes, they conduct blood to the periphery ; (ii) because they are elastic, they also serve as elastic capacitor of the pulsatile nature of flow from the heart.

This conversion phenomenon of the pulsatile pumping action into relatively smooth flow has been termed "Windkessel effect" by Otto Frank in 1899 (literally "Windkessel" = "air chamber") **[Sagawa *et al.*, 1990; Wang *et al.*, 2006]**. In fact, the Windkessel model considers the arterial system as a single compliant compartment with blood flowing into it from the left ventricle during systole. The modelisation provides a solution for the pressure of the arterial compartment, yielding an exponential function which increases during systole and falls during diastole **[Burkhoff *et al.*, 1988; Wesseling *et al.*, 1993]**.

To facilitate the investigation of ventriculo-aortic coupling, simplified models resembling the real arterial system have been developed. The most extensively used one is the Three-element Windkessel model (Figure 7).

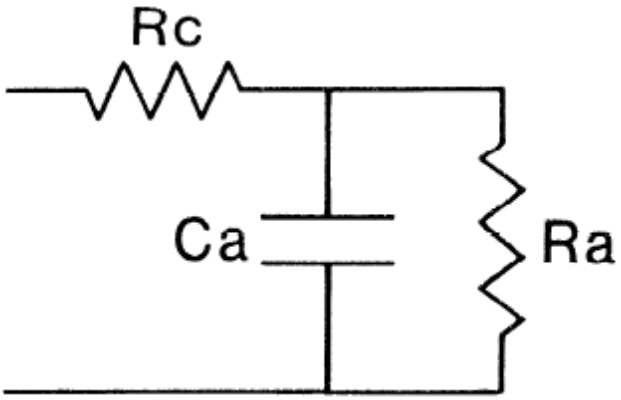

Figure 8. Three-element Windkessel model. Rc is the characteristic impedance; Ra is the peripheral arterial resistance; Ca is the arterial compliance. **[Burkhoff *et al.*, 1988]**). Rc is the component of vascular impedance wich depends directly to the viscoelastic properties, but do not include reflecion waves.

Wesseling **[Wesseling *et al.*, 1993]** made use of the Three-element model of arterial input impedance developed by Toorop **[Toorop *et al.*, 1987]** and Burkhoff **[Burkhoff *et al.*, 1988]** to obtain a reliable description of the relationship between aortic pressure and flow. Integration of the aortic flow over one heart beat provides SV. $\dot{Q}$ is then calculated as the product of SV times HR.

The three-element model on which the Modelflow® method relies was developed from the aortic pressure profiles. Yet its prevailing utilisation in humans is based on peripheral pressure profiles obtained from fingertips; thus allows to avoid aortic catheterization. However, the shape of peripheral pulse pressure profiles differs from that of aortic pressure profiles **[Remington *et al.*, 1956],** this induce a risk of inaccuracy**.**

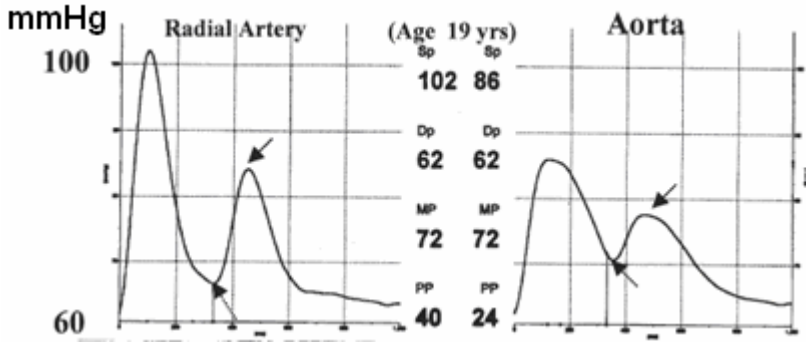

Figure 9. Differences in shape of peripheral and central artery pressure **[Nichols, 2005]**

Houtman **[Houtman *et al.*, 1999]** validated the Modelflow® method as applied to peripheral pressure profiles by performing a comparison of $\dot{Q}$ obtained by the CO_2 rebreathing method, with $\dot{Q}$ calculated simultaneously using Modelflow®, during steady state at moderate exercise levels. They observed a significant difference in the values of $\dot{Q}$ obtained from the two methods. They postulated that changes in sympathetic activity and total peripheral resistance could affect the calculation of SV from the pulse contour **[Houtman *et al.*, 1999]** obtained peripherally, since the impedance of aorta is not so affected. They concluded that $\dot{Q}$ computed by analysis of the contour of peripheral pulse pressures recorded non-invasively is inaccurate during exercise.

AIM OF THE THESIS

In this thesis, a validation of the Modelflow method for the assessment of $\dot{Q}$ was carried out. Two hypotheses were tested, namely: i) that the results of Houtman **[Houtman *et al.*, 1999]** were a consequence of peripheral recording of pulse pressure profiles, and : ii) that the demonstrated inaccuracy of Modelflow® is due to a systematic error, and thus can be corrected by an appropriate calibration procedure against an established steady-state method.

In order to test the first hypothesis, beat-by-beat $\dot{Q}$ values obtained by application of Modelflow® to pulse pressure profiles recorded non-invasively from a finger artery ($\dot{Q}_{porta}$) were compared with the beat-by-beat $\dot{Q}$ values obtained by application of the same model to the corresponding pulse pressure profiles recorded simultaneously from the radial artery ($\dot{Q}_{pia}$), at both rest and during exercise. This comparison was the object of the first study.

In order to test the second hypothesis, an independent method ($\dot{Q}$ measured by open-circuit acetylene technique, $\dot{Q}_{C_2H_2}$) was used to determine a calibration factor that can be used to perform appropriate corrections of average steady-state $\dot{Q}_{pia}$ values obtained by Modelflow® ($\dot{Q}_{Modelflow®}$) at rest and exercise. An analysis of this correction procedure was performed.

The results of these studies were published in two original papers **[Azabji Kenfack *et al.*, 2004; Tam *et al.*, 2004]**.

FIRST STUDY

1 METHODS

1.1 SUBJECTS

Seven healthy non-smoking young subjects took part to the experiments (age, 24.0 ± 2.9 years, and weight, 81.2 ± 12.6 kg). All subjects were informed about the procedures and the potential risks of the experiments and signed an informed consent form. The study was carried out after obtaining local ethical approval.

1.2 METHODS

An intra-arterial catheter (Seldi Cath 3F; Plastimed, St. Leu Lafôret, France) carrying a pressure head (Grass-Telefactor; Astro-Med, West Warwick, RI, U.S.A.) for continuous intra-arterial pressure recording was inserted into the left radial artery. The photoplethysmographic cuffs of a finger pressure device (Portapres®; TNO TPD Biomedical Instrumentation, Amsterdam, The Netherlands) were placed on the third and fourth fingers of the contralateral hand. The subject's arms were sustained by a scarf fixed around the neck, in order to avoid compression due to handlebar grasping. The gain of the intra-arterial pressure head was 100 mmHg/V, with a 0 mmHg signal set at 0 V (ambient air). The Portapres® signal was calibrated following the procedure indicated by the manufacturer. The height adjustment sensor and the reference were positioned according to the manufacturer's instructions. Pulse pressure signals from both devices and the ECG were digitized by means of a 16-bit A/D converter (MP100, Biopac Systems, Santa Barbara, CA, U.S.A.) operated by commercial software (ACK100W; Biopac Systems) running on a PC. Acquisition rate was set at 100 Hz. Exercise was performed on an electromagnetically braked cycle ergometer (Ergomed 840L; Siemens, Erlangen, Germany).

The pressure profiles were fed to a computer running the Beatscope® software program (version 1.0; TNO-TPD Biomedical Instrumentation), implementing the Modelflow® model **[Wesseling *et al.*, 1993]**. The pulse pressure profiles were analysed off-line to determine beat-by-beat systolic (P_s), diastolic (P_d) and mean (P_m) blood pressures. Beat-by-beat HR and SV were then calculated by using the procedure incorporated in the Beatscope® software. Beat-by-beat $\dot{Q}$ was then calculated as the product of SV times the corresponding HR.

2 PROTOCOL

After insertion of the arterial catheter, the subject sat on the ergometer and the Portapres® cuff was positioned. After 5 min of recording at rest, exercise at 50 W was started for 10 min. After a 10 min recovery, the 100 W exercise started, again for a 10 min duration, followed by a 10 min recovery. The pulse pressure profiles from both the intraarterial catheter and the Portapres® device were recorded continuously during the entire protocol.

3 STATISTICAL ANALYSIS

Data are presented as means ± S.D. Each mean value is the mean of all the beats at rest and at the steadystate exercise levels (from fourth min of exercise onwards) recorded on all subjects, *n* was 10048 for each parameter. The effects of the measurement site (radial artery compared with the fingertip) were evaluated by Student's *t* test for paired observations. Linear regression was calculated by the least-squares method using the procedure of Brace **[Brace, 1977]**. Agreement between the two methods of measurements was assessed by means of Bland–Altman analysis **[Bland *et al.*, 1986]**. Significance level was set at $p<0.05$.

4 RESULTS

An example of pulse pressure tracings recorded simultaneously at rest from the radial artery and from the finger is shown in Figure 9. Absolute pressure values are systematically higher in the radial artery than the finger. Moreover, the shape of the pressure profiles differed between the two recordings: the tracings from the fingertip showed a larger dicrotic incision and a more rapid pressure decrease during diastole.

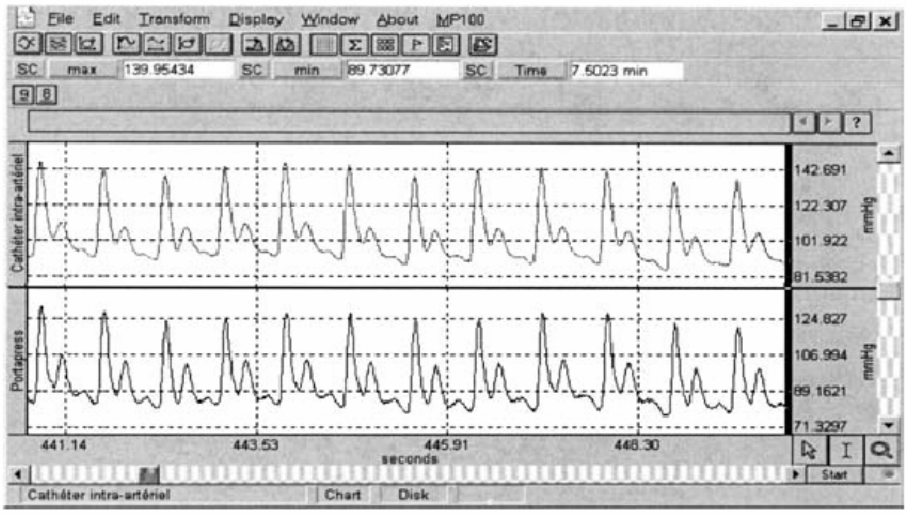

Figure 10. Example of intra-arterial (upper) and finger (lower) pulse pressure profiles recorded at rest

The mean values of P_s, P_d and P_m at rest and at the steady state of the two investigated workloads from both the intra-arterial pressure head and the Portapres® finger cuff are shown in Table 1. The values from the finger were significantly and systematically lower than those from the radial artery. In addition, the S.D. of P_s, P_d and P_m obtained from the Portapres® finger cuff, with the exception of resting P_s, were significantly ($p<0.05$) greater than those from the radial artery.

Table 1: Means P_s, P_d, P_m and $\dot{Q}$ at rest and exercise steady state (50 and 100 W) measured from the intra-arterial pressure head and the finger device (Portapres®). Values are ± SD. Each mean value is the result of 10'048 measurements.

		Intra arterial (pia)	**Fingertip (porta)**
P_s (mmHg)	*Rest*	152.4 ± 16.4	120.4 ± 19.3
	50W	175.5 ± 7.8	167 ± 33.9
	100W	191.5 ± 0.7	172 ± 24
P_d (mmHg)	*Rest*	96.5 ± 14.9	84 ± 36.8
	50W	88.5 ± 5	77.5 ± 33.2
	100W	87.4 ± 8.2	78.2 ± 12.6
P_m (mmHg)	*Rest*	122 ± 21.2	100 ± 36.8
	50W	116 ± 9.9	102 ± 39.6
	100W	113.7 ± 1.9	100.5 ± 15.7
$\dot{Q}$ (litre·min^{-1})	*Rest*	6.9 ± 0.6	6.6 ± 3.3
	50W	10.1 ± 1.2	11.2 ± 3.9
	100w	11.5 ± 1.9	13.4 ± 3.7

The corresponding mean values (n=10'048) of the values $\dot{Q}_{pia}$ and $\dot{Q}_{porta}$ are also shown in Table 1. The mean steady-state $\dot{Q}_{porta}$ values were significantly ($p<0.05$) and systematically higher than those for $\dot{Q}_{pia}$. In addition, as for P_s, P_d and P_m, the S.D. of $\dot{Q}_{porta}$ were systematically larger than those of $\dot{Q}_{pia}$. Figure 10 shown the $\dot{Q}_{porta}$ from all heart beats in all subjects as a function of the corresponding $\dot{Q}_{pia}$. The corresponding linear relationship is described by the following regression equation: $y=1.55x-3.02$, $R^2=0.640$.

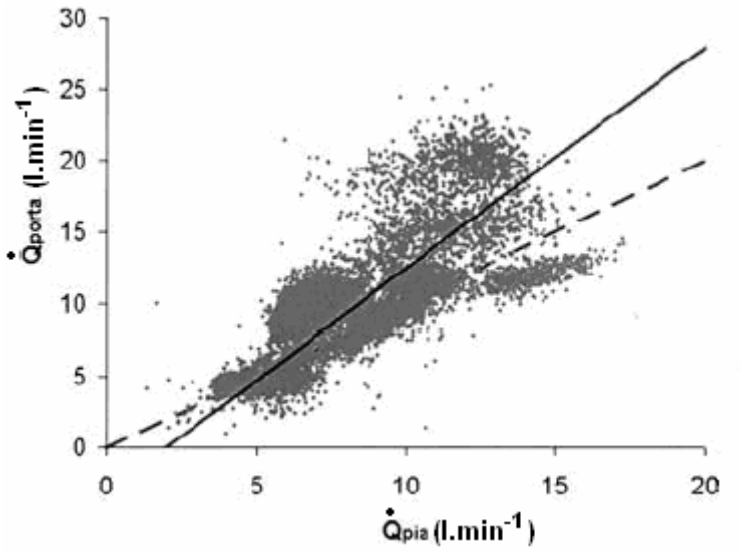

Figure 11. $\dot{Q}_{porta}$ as a function of $\dot{Q}_{pia}$.
All individual beats at rest and exercise are reported (n=10'048). The continuous line is the regression line, the broken line is the equality line.

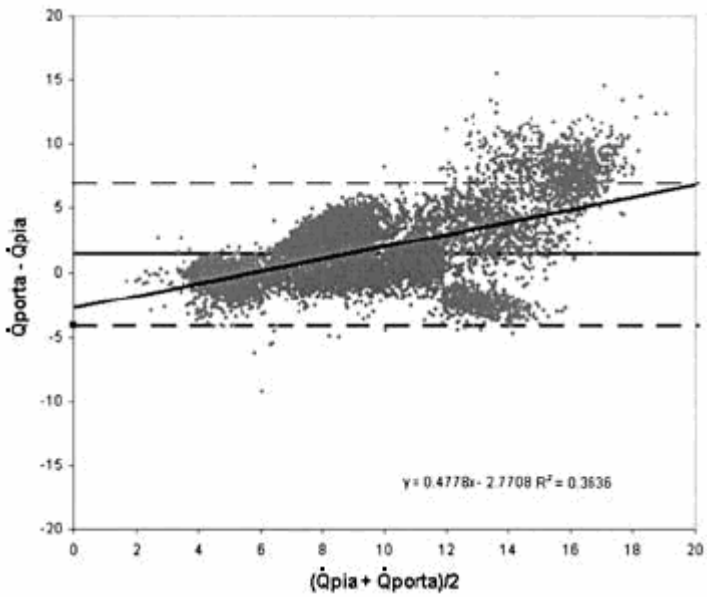

Figure 12. Bland–Altman analysis of the differences between $\dot{Q}_{porta}$ and $\dot{Q}_{pia}$ as a function of the average value of the two values for each beat.
The line relating these parameters has a significant positive slope (y=0.48 x −2.77 $l.min^{-1}$, [x]=1 $l.min^{-1}$, r=0.603).

The results of the Bland–Altman analysis are shown in Figure 11. The bias (mean of the difference $\dot{Q}_{porta} - \dot{Q}_{pia}$) was 1.44 litre min^{-1}, with S.D. (precision) of 2.84 litre · min^{-1} ; the 95% confidence interval ranged from -4.12 to +7.01 litre · min^{-1}. The line regression shown in Figure 11 has a significant positive slope (y= 0.48x-2.77, r=0.603). This indicates that the

slope of the regression line between the individual $\dot{Q}_{porta}$ and $\dot{Q}_{pia}$ is significantly greater than 1. Thus, $\dot{Q}_{porta}$ carries along a systematic error with respect to the reference value, $\dot{Q}_{pia}$, for $\dot{Q}$ > 5 l.min^{-1}.

5 DISCUSSION

Equivalent pulse pressure tracings from the finger and main arteries should provide equivalent $\dot{Q}$ values. If this were the case, the relationship between beat-by-beat $\dot{Q}$ values determined from pulse pressure profiles recorded from the finger and the corresponding beat-by-beat $\dot{Q}$ values from the same pulse pressure profiles recorded simultaneously from a more proximal artery (in the present case the radial artery) would be described the line of equality (the line on which both sets of data would lie if they were identical). However, the main finding of the present study is that the slope of the line relating beat-by-beat $\dot{Q}_{porta}$ to beat-by-beat $\dot{Q}_{pia}$ is significantly higher than that of the equality line, as demonstrated by the Bland–Altman analysis (Figure 11). This indicates that the pulse pressure profiles recorded from the finger yield an overestimate of beat-by-beat $\dot{Q}$ with respect to the corresponding values obtained from the radial artery. From the slope of the regression line reported in Figure 10, this overestimate appears to be 55%.

That fingertip pressure profiles provide unreliable $\dot{Q}$ values has been shown in a previous study **[Houtman *et al.*, 1999]** in which a comparison of $\dot{Q}_{porta}$ with $\dot{Q}$ determined by CO_2 rebreathing at rest and at the steady state of submaximal workloads was carried out. In that study the average difference (bias) between the two methods amounted to 2.27 ± 3.90 litre min^{-1}. In the present study, the comparison between $\dot{Q}_{pia}$ and $\dot{Q}_{porta}$ suggested that the inaccuracy reported by Houtman **[Houtman *et al.*, 1999]** could be due, at least partly, to an error persisting by the peripheral site of pulse pressure recording and not to the pressure pulse principe. Since we were not authorized to place a catheter in the aorta for the purposes of this study, the radial artery was retained as the site for invasive pulse pressure profile recordings, despite the fact that the Modelflow® makes use of constants determined on the *post mortem* elastic characteristics of the aorta **[Langewouters *et al.*, 1984]**. In fact the radial artery has the

typical structure of an elastic artery **[Laurent *et al.*, 1994]**, and the error introduced with respect to the aorta is expected to be negligible in the present context.

In addition, Wesseling **[Wesseling *et al.*, 1993]**, measured $\dot{Q}$ by Modelflow® from the radial artery, and concluded that the $\dot{Q}$ values obtained from this site were sufficiently accurate with respect to computations from the aorta, despite the changes in flow characteristics while moving from the latter to the former site. On these bases, the use of radial artery pressure profiles as a reference site in the present study seemed to be justified.

Non-invasive photoplethysmographic recordings of pulse pressure, are made on peripheral small arteries of the finger, and possess lower absolute pressure values **[Idema *et al.*, 1989; Gizdulich *et al.*, 1997]** as shown in Table 1, and a different pulse form **[Remington & Wood, 1956]** as indicated in Figure 9. The lower blood pressure values observed in the finger arteries may be explained (if real) either by the significant hydraulic resistance to flow with respect to more proximal arteries, or by the vascular tone prevailing at the finger level. If the former explanation is correct, different relationships between pulse pressure and flow may be expected when compared to the radial artery, and this may explain most of the significant differences between $\dot{Q}_{porta}$ and $\dot{Q}_{pia}$ observed in the present study.

If alternatively, the second explanation is correct, the answer would only be obtained after performing a similar experiment following administration of a blood pressure enhancer, such as phenylephrine.
The computation algorithm implemented in the Beatscope® software includes a waveform filtering procedure aimed at reconstructing the brachial artery pressure pulse from the finger arterial pressure, and a correction for pressure level is also introduced **[Bos *et al.*, 1996; Gizdulich *et al.*, 1997]**. This correction procedure improves the accuracy of the method by reducing the pressure level differences between the two sites. Nevertheless, the data in the present study show that these corrections are insufficient to provide equivalent values of $\dot{Q}_{porta}$ and $\dot{Q}_{pia}$, especially during exercise.

The so-called characteristic impedance (cZ) method is another earlier means of calculating $\dot{Q}$ from pulse pressure analysis **[Warner *et al.*, 1953; Kouchoukos *et al.*, 1970; Alderman *et al.*, 1972]**. Also this method was modified in order to take into account the effect of changes in blood pressure, vascular tone, HR and vascular resistance that occur during exercise,

hypervolaemia and application of lower body negative pressure contour **[Stok *et al.*, 1993; Antonutto *et al.*, 1994; Antonutto *et al.*, 1995]**. However, the corrections incorporated in the cZ method are based on empirical coefficients, whereas Modelflow®, although relying on the *post mortem* elastic characteristics of the major aortic vessels, exploits a theoretical model of the vascular system. Finally, the variations of resting $\dot{Q}$ with the cZ method turned out to be larger than with Modelflow® **[Stok *et al.*, 1993]**.

When only changes in $\dot{Q}$ with respect to baseline values are to be investigated, Modelflow® applied to non-invasive continuous recordings of pulse pressure profiles from the finger could be a reliable method for $\dot{Q}$ measurements. Because the error shown for $\dot{Q}_{porta}$ is systematic, the relative $\dot{Q}$ changes obtained with Modelflow® are probably sufficiently accurate, even though they are less reliable than those obtained from other established steady-state methods **[Stok *et al.*, 1993; Wesseling *et al.*, 1993; Houtman *et al.*, 1999; Remmen *et al.*, 2002]**. Absolute $\dot{Q}$ values computed from non-invasive pulse pressure profiles from the finger were found, however, to be significantly different from those obtained from the radial artery. Therefore, if radial artery pressure profiles provide accurate $\dot{Q}$ values **[Wesseling *et al.*, 1993]**, absolute non-invasive $\dot{Q}$ values with Modelflow® should not be used without correcting them with a calibration factor obtained previously by means of an independent established method. This conclusion is similar to that attained by others for the cZ method **[Stok *et al.*, 1993; Antonutto *et al.*, 1994; Antonutto *et al.*, 1995]**. In the accompanying paper (second study), the accuracy of Modelflow® applied to non-invasive pulse pressure profiles is analysed and a correction procedure proposed.

SECOND STUDY

1 METHODS

1.1 SUBJECTS

Experiments were carried out on nine male healthy subjects (age, 24.6 ± 2.96 years; body mass, 74.6 ± 6.90 kg; and height, 180.4 ± 4.03 cm). All subjects were informed about the procedures and the potential risks of the experiments and they all signed an informed consent form. The study was approved by the Ethics Committee of the School of Medicine of Udine and complied with the Declaration of Helsinki .

1.2 METHODS

Reference $\dot{Q}$ ($\dot{Q}_{C2H2}$) was measured by means of an open-circuit acetylene technique **[Barker *et al.*, 1999]**. At rest and steady state exercise, the subject inhaled a gas mixture containing 21% O_2, 6% helium and 1.5% acetylene balanced with nitrogen for a total of 20-25 breaths. Gas concentrations during breathing of the mixture were monitored continuously on a mass spectrometer (Airspec 2200, Gillingham, Kent, U.K.). Inspired and expired gas volumes were determined by an ultrasonic flowmeter (Tuba; GHG, Zurich, Switzerland). Gas fractions and flow signals were calibrated before each experiment by means of gas mixtures of known composition and by means of predefined inspiratory and expiratory volumes obtained by using a calibrated 3 litres syringe (Hans Rudolph, Kansas City, MO, U.S.A.). At each $\dot{Q}_{C2H2}$ measurement, a pneumatic piston operated a shuttle valve (Hans Rudolph) placed between the ultrasonicflowmeter and a two-way non-rebreathing valve. The pneumatic servomechanism (Burosoft, Udine, Italy) deviated the inflow from ambient air to the acetylene-containing gas mixture administered from a high-pressure gas cylinder via a Douglas bag. $\dot{Q}_{Modelflow®}$ was determined continuously at rest and during exercise from arterial pulse pressure profiles recorded non-invasively by using a Portapres® system (TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). The photoplethysmographic cuffs of Portapres® was positioned on the index and middle fingers. The Portapres® signal was calibrated following the procedure indicated by the manufacturer. The height adjustment sensor and the reference were positioned according to the manufacturer's instructions.

Pulse pressure, gas fractions and respiratory flow signals were digitized by means of a 16-bit A/D converter (MP100; Biopac Systems, Santa Barbara, CA, U.S.A.) operated by commercial software (ACK100W; Biopac Systems) running on a PC. Acquisition rate was set at 100 Hz. Exercise was performed on an electromagnetically braked cycle ergometer (Ergomed 840L; Siemens, Erlangen, Germany).

The pressure profiles were fed to a computer running the Beatscope® software program (version 1.0; TNO-TPD Biomedical Instrumentation), implementing the Modelflow® model **[Wesseling *et al.*, 1993]**. The pulse pressure profiles were analysed off-line to determine beat-by-beat systolic (P_s), diastolic (P_d) and mean (P_m) blood pressures. Beat-by-beat HR and SV were then calculated by using the procedure incorporated in the Beatscope® software. Beat-by-beat $\dot{Q}$ was then calculated as the product of SV times the corresponding HR.

2 PROTOCOL

The subjects were referred to the laboratory after a light meal consumed 2 h before. They were seated on the ergometer and the Portapres® cuffs were positioned. After the calibration procedures were completed, the mouthpiece and the nose-clip were placed in position and the acquisition of the gas fractions, flow and blood pressure signals was started. After 4 min rest, $\dot{Q}_{C2H2}$ was measured. Then, the subject started pedalling against a 50 W workload at a constant pedalling rate of 60 revs · min^{-1}. At the fifth minute of exercise, $\dot{Q}_{C2H2}$ was measured again. At the end of the measurement, the subject immediately stopped and rested for 5 min. A new exercise run was then performed by increasing the workload by 50 W. Two additional exercise steps, separated by 5 min of rest, were performed up to the highest workload of 200 W.

3 CORRECTION OF $\dot{Q}_{MODELFLOW®}$ WITH $\dot{Q}_{C2H2}$

In order to correct the $\dot{Q}_{Modelflow®}$ values by use of $\dot{Q}_{C2H2}$, a workload was selected and, at the steady state, the average $\dot{Q}_{Modelflow®}$ was calculated as the mean of beat-by-beat values over 1 min. A calibration factor was then calculated as the ratio of average $\dot{Q}_{Modelflow®}$ to average $\dot{Q}_{C2H2}$. The selected workload was 150 W, that is the workload at which this ratio showed the

lowest variability. The calibration factors were then used to recalculate average $\dot{Q}_{Modelflow®}$ at rest and at all the remaining workloads.

4 STATISTICS

Correlation between variables was calculated by the least-squares method using the procedure of Brace **[Brace, 1977]**. Regression parameters were analysed by using the procedures for the comparison of regression lines of the first kind **[Lentner, 1982]**. The significance of differences between average values in the different workload conditions were evaluated by means of two-way ANOVA **[Box *et al.*, 1978]**. Student's *t* test for 1-sample analysis was utilized to reject the hypothesis zero. Agreement between the two methods of measurements was assessed by means of Bland-Altman analysis **[Bland & Altman, 1986]**. ANOVA for repeated measurements was applied to identify significant differences between averages related to the different workloads **[Daniel, 1991]**.

5 RESULTS

The average $\dot{Q}_{Modelflow®}$ and $\dot{Q}_{C2H2}$ values are shown in Figure 12 as a function of the workload. Linear regressions were calculated on the entire data for both variables (n=45;

$\dot{Q}_{Modelflow®}$: $y = 0.092x + 7.09$ l.min^{-1}, where [x] = 1 W ; (r=0.926, p<0.001)

$\dot{Q}_{C2H2}$: $y = 0.10x + 4.41$ l.min^{-1}, where [x] = 1 W ; (r=0.937, p<0.001).

The two lines had thus practically the same slope, but significantly different y-intercepts. This means that $\dot{Q}_{Modelflow®}$ was shifted upward with respect to $\dot{Q}_{C2H2}$.

The ratios between $\dot{Q}_{Modelflow®}$ and $\dot{Q}_{C2H2}$ at the different workloads are shown in Table 2. These ratios varied with the workloads. The ratios at 100, 150 and 200 W were not significantly different from 1. The smallest S.D., and thus the smallest coefficient of variation (C.V = 100SD/Ratio), was found at 150 W. The average of all correction factors computed at all the workloads, including rest, was 0.87 (± 0.269).

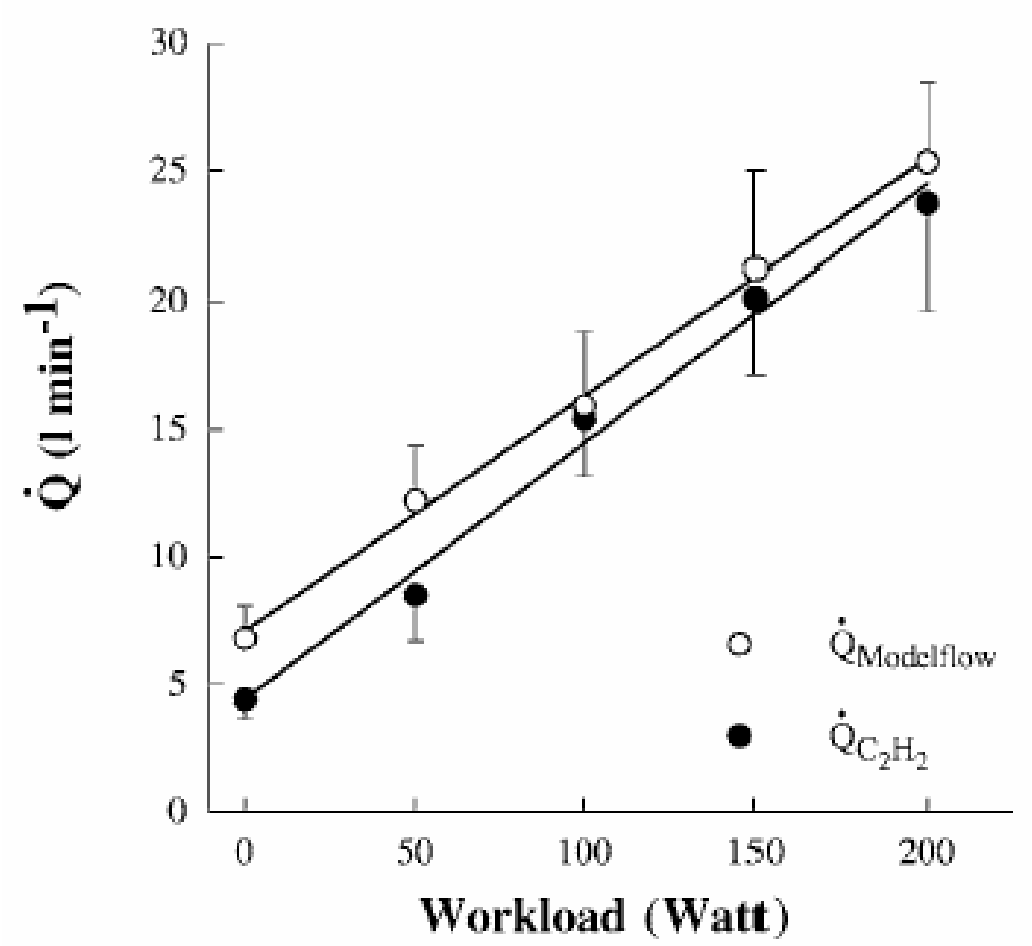

Figure 13. Mean values of $\dot{Q}_{Modelflow®}$ and $\dot{Q}_{C2H2}$ plotted against workload

Table 2: Means, S.D. and coefficient of variation (C.V) of $\dot{Q}_{C2H2} / \dot{Q}_{Modelflow®}$ ratios at rest and at the four workloads

Workload (W)	$\dot{Q}_{C2H2} / \dot{Q}_{Modelflow®}$		
	Ratio	**S.D**	**C.V (%)**
0	0.65	0.198	28.7
50	0.70	0.252	35.0
100	0.96	0.320	31.5
150	0.94	0.220	22.6
200	0.94	0.223	23.3

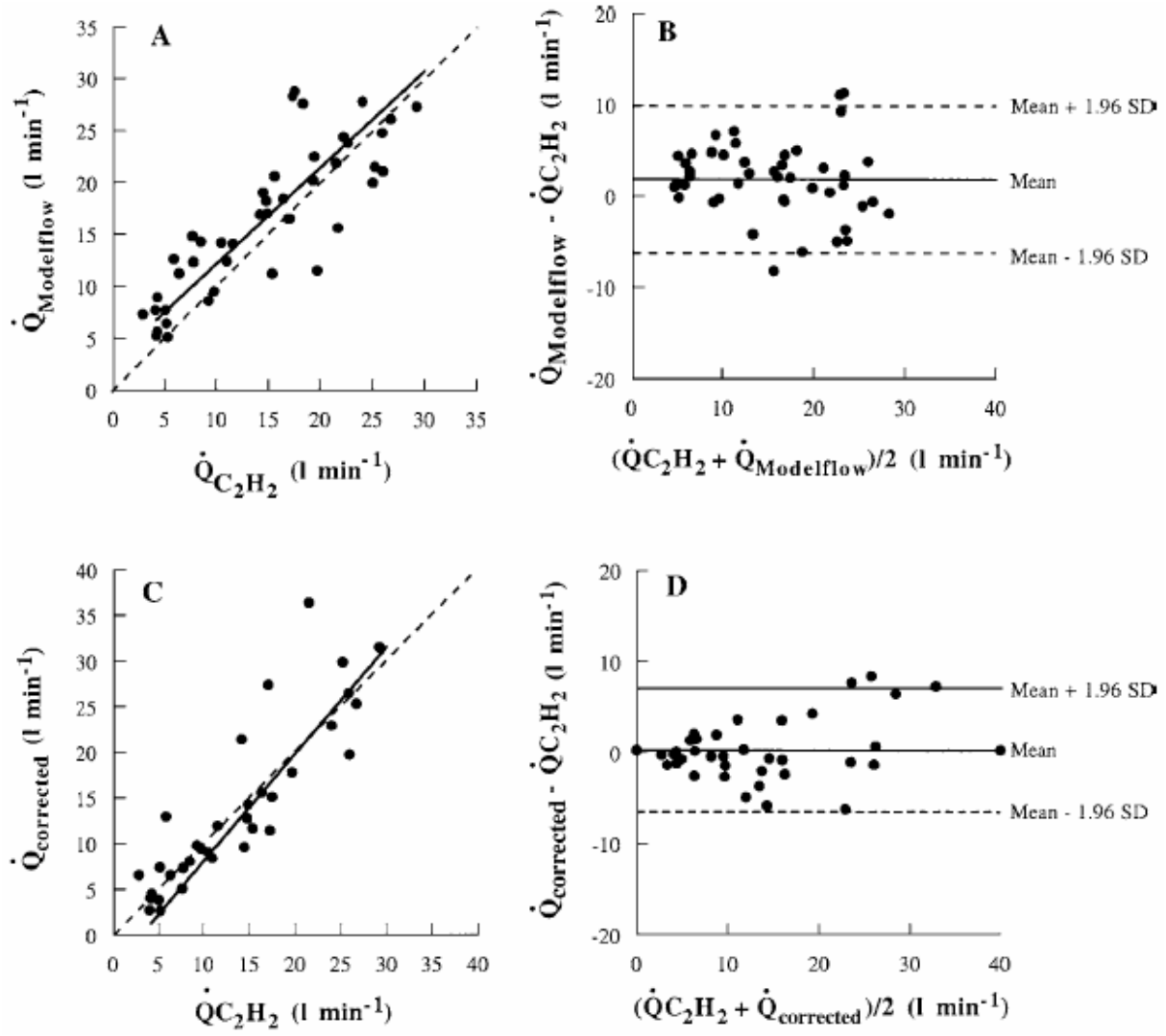

Figure 14. Relationship between $\dot{Q}_{Modelflow}$®, $\dot{Q}$C2H2 and $\dot{Q}_{Corrected}$

(A) $\dot{Q}_{Modelflow}$® determined for each subject plotted against the corresponding $\dot{Q}_{C2H2}$ values. (B) Difference between $\dot{Q}_{C2H2}$ and $\dot{Q}_{Modelflow}$® values plotted against their mean. (C) $\dot{Q}_{Corrected}$ values determined in each subject plotted against the corresponding $\dot{Q}_{C2H2}$ values. (D) Difference between $\dot{Q}_{C2H2}$ and $\dot{Q}_{Corrected}$ values plotted against their mean. In (A) and (C), the broken lines correspond to the lines of equality, and the solid lines are the regression lines. In (B) and (D), broken lines represent the 95 % limits of agreement.

Individual mean $\dot{Q}_{Modelflow^{®}}$ plotted as a function of the corresponding $\dot{Q}_{C2H2}$ are shown in Figure 13(A). The linear relationship between these two parameters was $y = 0.932x + 2.81$ l.min^{-1} ; [x] = 1 l.min^{-1}, indicating that the regression line was displaced upward with respect

to the equality line (the line on which both sets of data would lie if they were identical). The $\dot{Q}_{Modelflow®}$ values were significantly correlated with the $\dot{Q}_{C2H2}$ values (r=0.784, p<0.01).
The results of the Bland–Altman analysis are shown in Figure 13(B). The bias (mean of the differences $\dot{Q}_{Modelflow®} - \dot{Q}_{C2H2}$) was 1.83 litre · min^{-1}. The bias value was significantly larger than 0, thus confirming that the regression line was displaced upward with respect to the equality line. The S.D. (precision) was 4.11 litre · min^{-1} and the 95% limits of agreement ranged from -6.23 to +9.89 l.min^{-1}.

Corrected $\dot{Q}_{Modelflow®}$ values ($\dot{Q}_{Corrected}$) at rest and at all workloads, except 150 W which was used to calculate the correction factors (see the Methods section), are plotted as a function of the corresponding $\dot{Q}_{C2H2}$ in Figure 13(C). The regression equation was y =1.177x - 3.75 l.min^{-1}, [x]=l.min^{-1}. $\dot{Q}_{Corrected}$ values were correlated significantly with $\dot{Q}_{C2H2}$ values (r=0.931, p<0.01). The results of the Bland–Altman analysis are shown in Figure 13(D). The bias (mean of the differencences $\dot{Q}_{Corrected} - \dot{Q}_{C2H2}$) was 0.24 litre·min^{-1} and did not differ from 0. This indicated that the line relating $\dot{Q}_{Corrected}$ and $\dot{Q}_{C2H2}$ was equal to the equality line. S.D. (precision) was 3.48 litre · min^{-1} and the 95% limits of agreement were at -6.58 and +7.05 min^{-1}.

6 DISCUSSION

In the present study, the hypothesis that, after appropriate correction with an independent method, Modelflow® provides reasonably accurate $\dot{Q}$ values was tested at rest and during exercise. To this end, $\dot{Q}$ values obtained non-invasively with Modelflow® were corrected using independently established $\dot{Q}$ values measured with an open-circuit acetylene technique. The main result of the present study is that, after such a correction, Modelflow® applied to the arterial pulse pressure measured noninvasively on the finger provides a non-biased and reliable measure of $\dot{Q}$ in healthy subjects at rest and during exercise ranging from moderate to severe intensities. In contrast, uncorrected Modelflow® $\dot{Q}$ values ($\dot{Q}_{Modelflow®}$) are significantly different from the corresponding $\dot{Q}_{C2H2}$ values.

To our knowledge, the present study is the first in which $\dot{Q}_{Corrected}$ values were compared with those measured with a respiratory method ($\dot{Q}_{C_2H_2}$) during high intensity exercise at steady state in humans. As such, the method offers a valid non-invasive approach for the assessment of $\dot{Q}$ on a beat-by-beat basis that is applicable not only at the exercise steady state, but also during exercise transients.

The results presented in Figures 12, 13(A) and 13(B), showing uncorrected $\dot{Q}_{Modelflow^{®}}$ values, are consistent with those obtained by others. Remmen **[Remmen *et al.*, 2002]** compared Modelflow® applied to peripheral pulse pressure profiles with thermodilution and showed that Modelflow® did not yield accurate $\dot{Q}$ values in healthy elderly subjects at rest. Houtman **[Houtman *et al.*, 1999]** showed that Modelflow® did not accurately predict $\dot{Q}$ during cycling exercise of moderate intensity compared with the CO_2 rebreathing procedure. Taken together, these results underline the need for correcting $\dot{Q}_{Modelflow^{®}}$ values with a calibration factor obtained by an independent method. At rest, such a correction was shown to substantially improve the accuracy of $\dot{Q}_{Modelflow^{®}}$ values **[Jellema *et al.*, 1999]**. The improvement at rest subsisted when haemodynamic conditions were modified either pharmacologically or due to surgery **[Wesseling *et al.*, 1993]**. In none of the cited studies, however, was such a correction applied during exercise. This was done in the present study, and this is of novelty.

The $\dot{Q}_{C_2H_2}$ values assessed at 150 W were taken as the reference. The rationale of this choice was based on the results obtained from a post-hoc analysis performed on the $\dot{Q}_{Modelflow^{®}}$ / $\dot{Q}_{C_2H_2}$ ratios at the various workloads. This analysis showed that $\dot{Q}_{Modelflow^{®}}$ / $\dot{Q}_{C_2H_2}$ ratio at 150 W was closest to, and not significantly different from, 1 and had the lowest coefficient of variation.

The open-circuit soluble gas method **[Becklake *et al.*, 1962; Barker *et al.*, 1999; Johnson *et al.*, 2000; Bell *et al.*, 2003]** used for correction in the present study is a well-established method for $\dot{Q}$ computation. It showed fairly good agreement with the direct Fick method both at rest and during exercise up to 90% of maximal O_2 uptake ($\dot{V}_{O_2\,max}$) **[Becklake *et al.*, 1962; Barker *et al.*, 1999; Johnson *et al.*, 2000]**. A comparison of the open-circuit acetylene uptake with the closed-circuit acetylene rebreathing method at exercise was recently carried out **[Bell**

***et al.*, 2003]** and showed a very good agreement between the two methods. However, investigators are not compelled to use this method for the correction of $\dot{Q}_{Modelflow®}$ values: any steady-state method, either invasive or non-invasive, may be conveniently used, provided it is at least as accurate and precise as the respiratory method used in the present study.

The present study showed that $\dot{Q}_{Modelflow®}$ was significantly larger than $\dot{Q}_{C2H2}$. This is consistent with the results of the companion paper **(first study)** and with the data from Houtman, **[Houtman *et al.*, 1999]** during cycling exercise. This overestimate of $\dot{Q}$ is at least partially explained by the peripheral site of signal sampling, as demonstrated in the companion paper **(first study)**. It is noteworthy, however, that Modelflow® relies on data from the elastic properties of thoracic and abdominal aortas obtained from *post-mortem* examinations of patients 30- to 88-years old **[Langewouters *et al.*, 1984]**. The age of our subjects is below this range, thus introducing a further potential source of error. However, correction was carried out with a respiratory technique and this allowed circumvention of all the problems brought about by the assumption of the given elastic characteristics of the aorta.

In conclusion, it can be stated that Modelflow® applied to non-invasive recordings of pulse pressure profiles from small peripheral arteries can be considered a reliable procedure for measuring $\dot{Q}$ on a beat-by-beat basis in resting and exercising humans, provided that a correction by a well-established independent steady-statemethod (open-circuit acetylene uptake in the present case) is carried out. Therefore, applied in combination with such a correction, it could be accepted as an excellent alternative to invasive approaches for measuring $\dot{Q}$ in dynamic conditions and exercise transients both in healthy subjects and cardiovascular patients.

The need for an independent individual recalibration of the method, however, does not allow us to apply Modelflow® to the monitoring of large cohorts of patients in the clinical environment. Its utilization must be restricted to the study of specific, highly monitored situations, such as research protocols on a limited number of subjects, but only if access to a calibration procedure is possible.

ACKNOWLEDGMENTS

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