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Clinical Pharmacokinetics of Intravenous Ethanol

Relationship Between the Ethanol Space and Total Body Water

By

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Hofstadters lag:

“Det tar alltid längre tid än man tror, även när man tar hänsyn till Hofstadters lag.”

Douglas R Hofstadter.
Gödel, Escher, Bach: ett Evigt Gyllene Band.
Brombergs 1985

To my Family

ABSTRACT

Introduction: Total body water (*TBW*) is an important parameter in pathological states where the normal regulation of fluid balance is impaired (e.g., during critical illness, congestive heart failure, burn injury and renal insufficiency). Dilution of water isotopes, such as deuterium oxide (D_2O), is considered the gold standard method for measuring *TBW* in humans. However this procedure requires skilled staff, expensive equipment, and the results are seldom available in a timely fashion, which makes it less suitable for clinical applications. Ethanol is completely miscible with water and is thought to distribute into the *TBW*. The ethanol volume of distribution at steady state (V_{ss}) can be estimated by pharmacokinetic analysis of the concentration-time profile. Ethanol can be measured in expired breath with high precision and this non-invasive method of analysis could provide an attractive alternative with the prospect of bedside monitoring of V_{ss} within 4-6 hours.

The aim of this thesis was to develop an appropriate pharmacokinetic model for intravenous ethanol administration and to identify experimental factors that impact on the results. With this background, we compared V_{ss} determined by ethanol dilution with *TBW* determined by D_2O dilution. The complicated absorption kinetics of ethanol (e.g. variable gastric emptying and first-pass metabolism) was avoided by use of the intravenous route of administration.

Material and Methods: Forty-six healthy volunteers (20 women and 26 men) received intravenous infusions of ethanol (0.4-0.6 g/kg body weight) in 15-60 minutes. The concentration of ethanol was measured in end-expired breath by infrared spectrometry and in blood and urine by headspace gas chromatography. Specimens of blood and breath were obtained at 5-15 min intervals for 3-6 hours post-dosing. The concentration of D_2O in plasma-water was measured with isotope ratio mass spectrometry. Subjective feelings of inebriation were measured with a visual analogue scale.

A number of pharmacokinetic models were developed and evaluated in the course of this thesis. The effect of eating a meal on ethanol kinetics was investigated in crossover studies by giving the same dose of ethanol in fed and fasted states. The magnitude and time-course of arterio-venous differences in ethanol concentration were determined to assess the importance of local peripheral vasodilatation and vasoconstriction on results. This was achieved by warming the sampling hand in a heating box or cooling the hand in cold water during ethanol infusion experiments. The impact of sampling site on pharmacokinetic parameters of ethanol was investigated by simultaneous sampling in expired breath, arterial blood and venous blood. Inter- and intra-individual variations in precision of the estimates of V_{ss} and *TBW* were studied by making repeat infusions of ethanol a few days apart, and use of analysis of variance.

Results and discussion: The two-compartment model with parallel Michaelis-Menten kinetics and first-order renal elimination provided an excellent prediction of the entire concentration-time profiles of ethanol and proved to be theoretically superior to the other kinetic models tested. A good reproducibility was obtained for all pharmacokinetic parameters, especially for V_{ss} and the maximal metabolic rate (V_{max}).

Eating a meal increased the rate of ethanol metabolism by 30-60% and this confounding factor needs to be considered in clinical studies when V_{ss} for ethanol is estimated. Peripheral vasoconstriction caused a lowering of the concentrations of ethanol in venous blood, which also impacts on the pharmacokinetic parameters of ethanol. Measuring ethanol in breath agreed more closely with arterial blood concentrations than with venous blood. The pharmacokinetic

parameters of ethanol could be measured with equally high precision in expired breath and venous blood. An ethanol dose of 0.4 g/kg body weight infused in 15 minutes led to unacceptable inebriation in some volunteer subjects.

The precision of estimating V_{ss} by ethanol dilution was about the same as for measuring *TBW* by D_2O dilution (SD 0.8-1.1 litres). However, we observed a systematic bias of –13% between V_{ss} and *TBW* (D_2O). A likely explanation for this finding might be that water in the body, at least in part, is structured in such a way that ethanol is prevented from complete equilibration.

Conclusions: A two-compartment model with parallel Michaelis-Menten and first-order renal elimination gave an excellent fit to the concentration-time profile of ethanol after intravenous infusion. Feeding state and peripheral circulation affected the estimated model parameters. Sampling and analysis of breath provided results with the same high precision as venous blood for estimating ethanol V_{ss}. The non-invasive nature of breath sampling makes it more suitable for clinical purposes. The systematic bias of –13% between the V_{ss} and *TBW* by D_2O dilution suggests that ethanol does not distribute uniformly into the *TBW*. The reason for this discrepancy remains to be established.

LIST OF PAPERS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals I-V.

I **Eating a meal increases the clearance of ethanol given by intravenous infusion.**
Hahn RG, Norberg Å, Gabrielsson J, Danielsson A, Jones AW.
Alcohol and Alcoholism 1994; 29: 673-677.

II **Rate of distribution of ethanol into the total body water.**
Hahn RG, Norberg Å, Jones AW.
American Journal of Therapeutics 1995; 2: 50-6.

III **Pharmacokinetics of ethanol in arterial and venous blood and in end-expired breath during vasoconstriction and vasodilation.**
Norberg Å, Jones AW, Hahn RG.
American Journal of Therapeutics 1995; 2: 954-961.

IV **Within- and between-subject variations of pharmacokinetic parameters of ethanol by analysis of breath, venous blood and urine.**
Norberg Å, Gabrielsson J, Jones AW, Hahn RG.
British Journal of Clinical Pharmacology 2000; 49: 399-408.

V **Do ethanol and deuterium oxide distribute into the same water space in healthy volunteers?**
Norberg Å, Sandhagen B, Bratteby L-E, Gabrielsson J, Jones AW, Fan H, Hahn RG.
Alcoholism, Clinical and Experimental Research, 2001; 25: 1423-1430.

Reprints of the above articles were made with the kind permission of the publishers: Oxford University Press (paper I), Lippincott Williams & Wilkins (paper II, III and V) and Blackwell Science (paper IV).

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ABBREVIATIONS

Symbol	*Explanation*	*Unit*
^2H_2O	Deuterium oxide, see D_2O	
^3H_2O	Tritiated water, radioactive isotope of water	
A_i	Constant in the hybrid pharmacokinetic model, distribution	(amt/vol)
α	Distribution rate constant (paper II and III)	(1/time)
ABAC	Arterial blood alcohol concentration	(amt/vol)
ADH	Alcohol dehydrogenase	
AER	Alcohol elimination rate	(amt/time)
ALDH	Aldehyde dehydrogenase	
ANOVA	Analysis of variance	
AUC	Area under the concentration-time curve	(time • amt/vol)
B	Constant in the hybrid pharmacokinetic model (equal to C_0)	(amt/vol)
β	Elimination rate constant (paper II and III, equal to k_0)	(amt/(vol • time))
BBR	Blood:breath ratio	
BF	Body fat	(amt/amt)
B-Hb	Blood haemoglobin	(amt/vol)
BIA	Bioimpedance analysis	
BIS	Bioimpedance spectroscopy	
BMI	Body mass index	(kg/m^2)
BrAC	Breath alcohol concentration	(amt/vol)
bw	Body weight	(amt)
C	Concentration in the central compartment	(amt/vol)
C_0	Extrapolated concentration at time zero	(amt/vol)
c_0	Widmark's extrapolated concentration at time zero	(amt/amt)
CI	Confidence interval	
Cl_d	Intercompartmental distribution parameter, compare flow	(vol/time)
CL_{int}	Intrinsic clearance, the hepatic blood flow independent enzymatic capacity	(vol/time)
CL_H	Hepatic clearance	(vol/time)
CL_R	Renal clearance	(vol/time)
C_{max}	Maximum concentration	(amt/vol)
C_T	Concentration in the tissue compartment	(amt/vol)
CT	Computer tomography	
CV	Coefficient of variation	
D_2O	Deuterium oxide, stable isotope of water	
df	Degrees of freedom	
DXA	Dual energy X-ray absorption	
ECW	Extracellular water	(vol)
E_H	Extraction fraction across the liver	
F	Female	

fB_{water}	Fractional blood water content	(vol/vol) or (amt/amt)
FFM	Fat free mass	(amt)
F_H	Availability across the liver	
FPM	First-pass metabolism	
GC	Gas chromatography	
$H_2{}^{18}O$	Water with stable isotope of oxygen	
ICU	Intensive care unit	
ICW	Intracellular water	(vol)
IR	Infra red	
iv	Intravenous	
IVNAA	In-vivo neutron activation analysis	
k_0	Elimination rate constant in zero-order model	(amt/(vol • time))
K_m	Michaelis-Menten constant, the substrate concentration corresponding to 50% of the maximal metabolic elimination rate	(amt/vol)
K_{mH}	Michaelis-Menten constant, the substrate concentration corresponding to 50% of the maximal metabolic elimination rate, estimated for the liver as a separate compartment	(amt/vol)
LBM	Lean body mass	(amt)
M	Male	
MEOS	Microsomal ethanol oxidizing system	
MRI	Magnetic resonance imaging	
MRT	Mean residence time	(time)
MS	Mean square	
NAD	Nicotinamide adenine dinucleotide	
NADH	Reduced nicotinamide adenine dinucleotide	
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced	
NII	Near-infrared interactance	
n.s.	Not significant	
p	Widmark symbol for body weight (see *bw*)	(amt)
P	Partition coefficient for ethanol	
po	Per oral	
ppm	Parts per million	
Q_H	Liver blood flow (or liver blood water flow)	(vol/time)
r	Widmark factor corresponding to ratio of ethanol concentration in whole body to that in the whole blood	(amt/amt)
r_w	Water concentration ratio between the whole body and the blood	(amt/amt)
ρ	Tissue specific resistivity	
SD	Standard deviation	
SEM	Standard error of the mean	
TBW	Total body water	(vol)
UAC	Urine alcohol concentration	(amt/vol)
V	Variance	
VBAC	Venous blood alcohol concentration	(amt/vol)

V_C	Volume of the central compartment	(vol)
V_d	Volume of distribution	(vol)
V_H	Volume of the liver compartment	(vol)
V_m	Maximal metabolic rate constant derived from the integrated form of the Michaelis-Menten eauation.	(amt/(vol • time))
V_{max}	Michaelis-Menten constant, maximum metabolic elimination rate	(amt/time)
V_{maxH}	Michaelis-Menten constant, maximum metabolic elimination rate estimated for the liver as a separate compartment	(amt/time)
vs.	Versus	
VSAC	Venous serum alcohol concentration	(amt/vol)
V_{ss}	Volume of distribution at steady state	(vol)
V_T	Volume of the tissue compartment	(vol)
Z	Impedance	

INTRODUCTION

Body composition

There are many ways to describe body composition. Wang *et al.*[1] summarized the numerous multi-compartment models as one comprehensive model with five levels of increasing complexity: atomic, molecular, cellular, tissue system, and whole body. In this five-level model of body composition each component at successively higher level was composed of components from the lower levels. The molecular level can be divided into five compartments: fat, water, proteins, glycogen and minerals. However, a simple two-component model with body fat (*BF*) and fat-free mass (*FFM*) as the two components is often used. *FFM* contains about 73.2% water in most human beings,[2] and this observation has recently been reviewed.[3] Accordingly, it is possible to assess total body water (*TBW*) indirectly by any method capable of measuring *BF* or *FFM*, such as hydrodensitometry, dual energy x-ray absorptiometry (DXA) or total body potassium, provided the hydration of *FFM* is assumed to remain constant. *FFM* is the whole body excluding the mass of extractable fat, whereas lean body mass (*LBM*) is an anatomical term that refers to the body mass other than adipose tissue visible to the eye.[4]

Several review articles have dealt with body composition in humans and the methods used to estimate the separate components thereof.[5-10] Weighing of organs and chemical analysis of whole human cadavers provides the most accurate measure of body components. However, the availability of such data is limited. Some methods of measuring body composition are evaluated against an established reference method. For example, anthropometry and bioimpedance include errors from the reference methods used. Another type of body composition method, sometimes called *in-vivo* reference standards includes technical and model errors. Models based on chemical relationships, such as total body nitrogen/total body protein = 0.16, are often very stable. Models developed with nonchemical relationships such as *TBW*/*FFM* = 0.732, are less stable and are often influenced by disease, pregnancy or age.[7]

TBW can be divided into extra-cellular water (*ECW*) and intra-cellular water (*ICW*). The former is often measured by bromide dilution and the latter is obtained as the difference between *TBW* and *ECW*. In the intensive care unit (ICU) the validity of *ECW* measurement is uncertain, as there might be impairment of membrane patency to bromide. By estimating total body potassium, *TBW* and bromide space simultaneously in ICU-patients unrealistic values of intracellular potassium were obtained, suggesting that in severe illness the bromide dilution space no longer relates to *ECW*.[11] However, the bromide dilution method is still widely used in the ICU setting.[12, 13]

Total body water in the clinical setting

Total body water is a dynamic factor well regulated in healthy individuals. A decrease by 15% caused by dehydration can be life threatening. The turnover rate of *TBW* in a temperate environment is about 7% per day,[14, 15] but the individual variation can be huge. Normally, about 2 litres of water are lost per day with the urine and 800 mL to the breath, whereas losses with sweat, faeces and transdermal evaporation are negligible.[14] A host of factors influence the state of hydration such as physical activity, feeding state, meal composition, intake of fluids, environmental temperature and the menstrual cycle. The subjects are assumed to be in a stable state of hydration prior to assessment of *TBW* or *FFM*.

There are several situations in clinical medicine when a reliable measurement of a person's *TBW* would provide useful information. For example, in pathological states where the normal water balance is disturbed, such as in renal failure, congestive heart failure, burn injuries and critically ill patients in the intensive care unit (ICU). To some degree fluid balance can be assessed from a person's body weight (*bw*), or from arterial blood pressure and invasive measurements of filling pressures to the heart. Other ways include the chemical analysis of blood, such as osmolality, haematocrit, and sodium content as well as the volume and sodium content of the urine. Information about the central blood volume can be obtained by echocardiography. Just by recording a person's body weight one fails to distinguish whether changes are the result of hydration or catabolism of proteins. In terminal renal failure dialysis is guided by dry weight, but changes in body composition over time make adjustments necessary and a reliable estimate of *TBW* would be helpful.

Methods of measuring total body water - a review

The ideal method of measuring *TBW* should be inexpensive, accurate, precise, easy to repeat and use, non-invasive, and rapid enough to allow continuous bedside monitoring. Isotope dilution methods with D_2O, ^3H_2O or $H_2{}^{18}O$ as tracers are generally considered as the gold standard for assessing *TBW*.[7, 16] Use of ^3H_2O for *TBW* measurement introduces a minor radiation hazard and analysis of stable isotopes of water requires the use of expensive mass-ratio spectrometry equipment. Making repeat measurements necessitates increasing the dose administered because of accumulation of the tracer. However, the precision of isotope dilution methods is good, and these techniques are widely used for research purposes, but are not so practical in a clinical setting. Bioimpedance analysis (BIA) is a relatively new way of estimating *TBW* and is inexpensive and easily repeatable. However, BIA requires reference to another standard *TBW* method and also rests on certain assumptions some of which are either not thoroughly investigated or are known to be unreliable.[5, 7] Stray capacitance and variable resistance at the electrodes reduces the precision and value of this method in the complicated environment of the ICU.[17]

The availability of an ethanol dilution method would be a valuable complement to traditional methods. Ethanol is cheap and readily available in pure form and reliable analytical methods exist for quantitative determination of ethanol in body fluids.[18] Moreover, the concentration of ethanol can be determined in expired breath, which offers the possibility for making "low-invasive" measurements and bedside monitoring of patients. The rapid metabolism of ethanol introduces the need for repeated measurements and the use of an appropriate pharmacokinetic model. By using a rapidly metabolised tracer accumulation of tracer is avoided even if the measurement is repeated daily.

Direct methods

Desiccation

The direct methods of measuring *TBW* are completely independent of other methods or any underlying assumptions. Fresh carcasses can be heated and all losses in weight are assumed to represent body water. Some pioneer studies in the 19th and 20th centuries made use of human carcasses to estimate *TBW*.[19-26] (Table 1). However, the method is still widely used for animal carcasses.

Table 1
Total body water in adult humans by direct measurements in 11 cadavers.

Gender/Age	*bw (kg)*	*TBW (L/kg)*	Method	Comments	Reference
M 33	69.7	0.585	Desiccation	Executed, "healthy"	Bischoff, 1863 [19]
M 35	70.5	0.679	Desiccation	Congestive heart failure	Mitchell, 1945 [20]
M 25	71.8	0.618	Desiccation	Uraemia	Widdowson, 1951 [21]
F 42	45.1	0.560	Desiccation	Drowned, thin	Widdowson, 1951 [21]
M 48	63.8	0.815	Desiccation	Endocarditis, oedema, wasted	Widdowson, 1951 [21]
M 46	53.8	0.552	Desiccation [1]	Skull fracture	Forbes 1953 [22]
M 60	73.5	0.514/0.506	Desiccation [1]	Heart attack	Forbes 1956 [23, 24]
M 48	62.0	0.708	Desiccation [1]	Black, endocarditis	Forbes 1956 [24]
F 67 [2]	43.4	0.734	Desiccation	Advanced malignancy, wasted	Moore, 1968 [25]
M 63	58.6	0.597	Freeze drying	Oesophageal cancer	Knight 1986 [26]
F 59 [2]	25.9	0.512	Freeze drying	Advanced malignancy, cachectic	Knight 1986 [26]
47 ± 14	58.1 ± 14.9	0.62 ± 0.094			

[1] Performed at 65 ºC in a vacuum oven

[2] The cadavers of the 67 and 59 years old females were also analysed *in vivo* by ^3H_2O dilution 32 days (*bw* 46.2 kg, *TBW* 0.662 L/kg) and "a few" days before death (*bw* 25.2, *TBW* 0.619 L/kg), respectively. The large difference in water content in 59 years old subject elucidates the difficulties of the dilution method in the clinical situation.

Possible errors include evaporation of organic compounds,[27] water bound to proteins in such a way that it is not removed by desiccation,[28] measurement error in weighing, and too short desiccation time to reach constant weight.[29]

Lyophilization or freeze drying

In this method the carcass of the animal is weighed and then thoroughly homogenised. Samples of the homogenate are then used for the lyophilization procedure and the weight difference taken as the water content of the sample. Freeze drying has been validated against desiccation.[27] Errors are introduced if a non-representative sample is lyophilized or if water is bound to proteins in such a way that it remains even after the drying process is complete. Other possible errors arise from weighing, too short drying times, and loss of water or other substances during the initial preparation procedures.

Dilution methods

The simple relationship volume = mass/concentration can be used to assess the body fluid volume by the dilution principle. A tracer is diluted and well mixed in a solvent and the concentration of tracer then determined in a representative sample taken from the fluid. A variety of tracers have been used to measure different body fluid volumes in humans, such as the Evans Blue dye or ^{125}I-albumin for the plasma volume, and bromide for the extra cellular water.

An ideal tracer should be distributed only in the volume to be measured, it should be evenly distributed in that volume, the rate of equilibration should be rapid and the tracer should not undergo metabolism during the time of equilibration. Not many tracers fulfil all these requirements and adjustments are often needed; e.g. to correct for distribution outside the

volume space being investigated, to compensate for an uneven distribution of tracer by means of an appropriate pharmacokinetic model, and to compensate for losses of volume or tracer. Also other factors such as ready availability of tracer, purity and cost need to be considered.

Water isotopes

The discovery of deuterium (^2H_2O or D_2O) and tritium (^3H_2O) in the 1930s[30, 31] was soon followed by reports of isotope dilution analysis to estimate *TBW* in humans[32-34] (Table 2). Dilution of water isotopes has emerged as the gold standard for measuring *TBW* in humans. Three isotopes of water are available for this purpose: D_2O (^2H_2O), ^3H_2O and $H_2{}^{18}O$. Several animal studies have validated water-isotope dilution methods against lyophilization[35] and desiccation.[36-39] The unexplained difference between freeze-drying and isotope dilution in dogs[35] illustrates the difficulties that sometimes arise despite use of modern equipment and careful experimental design. However, the majority of animal studies report a very close relationship between *TBW* measured by water isotope dilution and *TBW* by desiccation.[29] It is difficult to see any major flaw in the concept of water-dilution methods. One source of error stems from the existence of non-aqueous hydrogen atoms in the body that equilibrate with the tracers resulting in an overestimation of *TBW*. The theoretical maximal size of this hydrogen pool has been estimated as 5.2% of *TBW* based on hydrogen content and turnover rate of different tissues.[40] Commonly used figures for the ratios of the water spaces to *TBW* are 1.041 for D_2O and 1.007 for $H_2{}^{18}O$.[16]

Tritiated water, ^3H_2O, is easy to measure by scintillation counting, and was the most commonly used isotope for several decades. However, the radiation hazard makes it less suitable than the other two water isotopes. Deuterium oxide, D_2O or ^2H_2O, is a non-radioactive isotope of water. However, analysis of deuterium requires use of rather complicated mass spectrometric equipment, which is expensive and requires highly skilled operators. Because of the relatively low mass to charge ratios, specimens cannot be analysed directly by mass spectrometry with any acceptable precision. Isotope fractionation occurs in water vapour relative to water fluid because of different energies or kinetic properties of the water isotopes that can lead to errors in the ratio of $^2H/^1H$.[41]

The Nobel Prize winner Georg von Hevesy first measured ^2H_2O by the falling drop method,[32] but later on several other methods were developed including mass spectrometry,[42] gas chromatography,[43] infrared spectrometry,[44] and nuclear magnetic resonance.[45] Today most methods rely on isotope mass ratio spectrometry because this offers the highest precision and very little material is needed for each analysis.[46] By this method the ratio between ^1H and ^2H is measured instead of an absolute value of ^2H. The third isotope $H_2{}^{18}O$ is also best analysed with mass ratio spectrometry.[47]

None of the isotopes of water fulfil all requirements of an ideal tracer as noted above. The slight overestimation of *TBW* can however easily be corrected for, and there is no evidence of an uneven distribution within *TBW*. The time to reach complete equilibration requires at least 3 hrs, and sometimes longer in situations with low cardiac output[48] or in fluid overload as often seen with ICU patients.[49] Water isotopes are not metabolised, and once equilibrium is developing, losses of ^1H and ^2H are likely to be very closely related. However, if daily measurements are required the dose must be increased to maintain a high precision because of accumulation of tracer owing to the slow turnover of body water.

The water isotope dilution method requires that the subject fasts overnight before specimens are taken for baseline measurements. The bladder is then emptied and an accurately

measured dose of the tracer is given by weighing the dosing device before and after administration. There are two ways to continue from here. In the plateau method fasting continues and samples of blood or urine are taken after 3 and 4 hrs. If the values from these two measurements are within ± 2 SD of the applied method the estimation is reliable and if not it might be best to discard them. In the slope method the subjects are allowed normal intake of food and nutrients but the sampling period is prolonged to several days or even weeks. The subjects are urged to refrain from heavy physical activity. The *TBW* is then estimated from the slope of the decay curve. This method is used in studies of metabolism with double-labelled water.[50] The half-life of deuterium oxide in the adult human body is approximately 9 days.[51] The complexity of the method of stable isotope dilution precludes its use for monitoring changes in *TBW* at the bedside. The long sample preparation time and the limited availability of the necessary mass spectrometric technique forces most investigators to send their specimens to another laboratory.

Potential errors of the water isotope dilution method include errors in the assumptions regarding exchangeable hydrogen, too short equilibration time, residual urine, preparation errors, dosing errors and error of analysis. If carefully conducted the precision can be as high as 1% coefficient of variation[5, 14] being among the highest of any body composition method.

Urea

Urea is a water-soluble major end product of protein metabolism in the liver. Serum normally contains 3-10 mmol/L urea and about 0.6 mmol is excreted in the urine daily. Variability is related to dietary intake of proteins, catabolic state, and diuresis. Urea is routinely analysed during care and treatment of patients with chronic renal failure to assess *TBW* and the need for dialysis.[52] Although single compartment kinetic models have been used for urea giving volumes of distribution close to *TBW*, compartmentalisation of urea during haemodialysis has been proposed,[53] and later confirmed by micro dialysis.[54]

The use of isotope labelled urea as a tracer for *TBW* is limited by multi-compartment kinetics, the rate of endogenous production, and the variable renal elimination. The coefficient of variation (CV) of the urea space has been estimated to be 7%.[55] Dilution of ^{14}C-urea is used as a method to determine tissue water.[56]

Antipyrine

Antipyrine is an analgesic drug developed in the 19th century and exhibits 100% bio-availability of the dose, a very low binding to plasma proteins (10%) and an apparent volume of distribution closely related to *TBW*. The metabolism of antipyrine follows first-order elimination kinetics with a half-life of approximately 11 hrs.[57, 58]

The variable plasma protein binding limits the usefulness of antipyrine as a tracer for measuring *TBW* and the long half-life results in accumulation of tracer with repeated dosing. As recently as 1996, however, antipyrine was reported as a suitable and accurate measure of total body water in haemodialysis patients.[57]

Sulphanilamide[59, 60] and *thiourea*[61] have also been proposed as markers for *TBW*, but both have been rejected because of unequal distribution in body tissues.[62-64]

Ethanol

Oskar Grüner first proposed ethanol dilution for determination of total body water in 1957,[65] and this methodology will be described in detail.

Table 2
Total body water in adult subjects by various indirect methods, summarized from the literature.

n, Gender, Age	*bw* (*kg*)	*TBW* (*L/kg*)	Method	Comments	Reference
Male	68	0.63	^2H_2O dilution	Normal subject	Hevesy, 1934 [32]
Male, 26 y	66.0	0.725	^2H_2O dilution	Normal subject	Moore, 1946 [33]
Male, 19 y	70.8	0.647	^3H_2O dilution	Normal subject	Pace, 1947 [34]
17 Males, 25 ± 4 y 11 Females, 25 ± 3 y	71.8 ± 6.6 58.2 ± 8.9	0.618 ± 0.037 0.519 ± 0.047	^2H_2O dilution ^3H_2O dilution	Normal subjects	Schloerb, 1950 [51]
7 males, 3 females	90.6 ± 33.9	0.510 ± 0.110	$H_2{}^{18}O$ dilution	5 normal, 5 obese	Schoeller 1980 [47]
6 Males 2 Females	60.5 ± 9.3 45.0 / 73.4	0.545 ± 0.030 0.495 / 0.393	Antipyrine dilution vs. ^2H_2O	Normal subjects	Soberman, 1949 [58]
9 M , 7 F, 58 ± 18 y	64.6 ± 9.0	0.68 ± 0.10	Antipyrine vs. ^3H_2O	Haemodialysis patients	Odar-Cederlöf, 1996 [57]
10 Males	71.8 ± 8.7	0.526 ± 0.055	Ethanol dilution	Normal subjects	Grüner, 1957 [65]
20 Males 34 patients (19F)	80.6 ± 13.2 64.6 ± 14.5	0.578 ± 0.049 0.588 ± 0.097	Bioimpedance vs. ^2H_2O	Patients in different degrees of hydration	Hoffer 1969 [66]

Bioimpedance and anthropometry

Bioimpedance

Bioelectrical impedance analysis (BIA) is based on the different conductive and dielectric properties of various biological tissues when an electric current is applied. Two underlying assumptions of the method are that the body behaves as a uniform isotropic conductor of electricity (isotropic = having like properties in all directions, like a piece of glass), and that the body can be viewed as a single cylinder. For the single frequency method, the volume or *TBW* is directly related to the specific resistivity of the tissues (ρ), the square of body stature, and inversely to the impedance (Z).[67, 68]

By using a large number of frequencies, typically logarithmically distributed between 1 kHz and 1000 kHz, some of the problems with single frequency bioimpedance are corrected.[69] This method is called bioimpedance spectroscopy, and several instruments are now commercially available. However, there are still several shortcomings of the impedance method in clinical practice.[70, 71]

Bioimpedance is a method with rather high precision but poor accuracy. The error of electrode placing is about 1%. Its ability to quantify changes of hydration is limited,[72] and if peritoneal dialysis fluid is added there is a severe underestimation of these volumes.[73] This is partly a consequence of the limb dominance of whole body impedance.[74] Factors like surgical shunts for dialysis and central venous lines have been shown to disturb measurements,[75] and there are problems when this method is used in the ICU because of stray capacitances to the environment.[17] Thus, bioimpedance serves best as a supplement or substitute to anthropometry in epidemiological field studies.

Anthropometry

Total body water can be estimated from easily available data such as length, weight, age and gender.[76] More sophisticated models can be made by adding skin fold thickness at various sites, bone dimensions, and limb circumferences.[6] The method requires a reliable reference method (e.g. isotope dilution) for comparison. A stepwise linear regression equation is fitted to a large group of individuals representative of the population under study. The reliability of the anthropometric method can never be better than the reference method and it is also difficult to find general equations that account for pathological states. Some of the well-known sources of error include profound malnutrition, inclusion of those with abnormal muscular mass (e.g. body builders), pregnancy, obesity and subcutaneous oedema.[77] In haemodialysis patients a population-specific regression equation has been developed to estimate *TBW*, but it has only been validated in 33 subjects by comparison with D_2O dilution.[78] Like bioimpedance, the anthropometric method is suitable for epidemiological field studies.

Methods of measuring body fat

The relationship $TBW = 0.732 \cdot FFM$ makes any method that can accurately measure *BF* a possible indirect measure of *TBW*. These methods are briefly listed below with focus on sources of errors, precision and accuracy.

Densitometry

Densitometry is a procedure for estimating body composition from body density. The most widespread method of densitometry is underwater weighing or hydrodensitometry.[79] Body mass is easily estimated from body weight, and therefore, most densitometry techniques are methods to measure body volume. *BF* can be calculated from whole body density by assuming that body fat has a density of 0.9 kg/L and *FFM* 1.1 kg/L.[80] The residual volume of the lungs and air in the gastrointestinal tract are two important sources of error. Even more important, however, is the magnitude of the deviation from the assumed fat-free composition.[81-83] The underwater weighing method is limited to subjects that can be totally submerged in water after a maximal expiration. The precision of *FFM* is about 2% and the accuracy 3%.[5] In recent years air displacement plethysmography, where the subject is immersed in a closed air-filled chamber, has begun to replace underwater weighing.[84, 85] Obviously this approach is not feasible for intensive care patients.

Dual energy X-ray absorptiometry (DXA)

Absorptiometry is the measure of an absorbed or attenuated radiation by the skeleton or soft tissues while an energy beam is transmitted through a subject.[86] High-resolution images, improved precision and lower radiation exposure of DXA has increased the availability of this technique. The fat content of soft tissue can be estimated by DXA from the assumed constant attenuation of pure fat and bone free lean tissue, respectively. It is *BF* rather than adipose tissue that is measured. The assumption of constant hydration of lean tissues has been pointed out as one source of error.[87, 88] Another problem is the thickness and size of the subject. Total body bone mineral is estimated independent of soft tissues by DXA. The precision of *BF* by DXA is 3% and accuracy 5-10% corresponding to a minimum detectable difference of 1 kg.[5]

Total body potassium

Potassium is essentially an intracellular cation that is not incorporated in triglycerides. The natural abundance of 0.012% of the radioactive isotope ^{40}K promotes estimation of body cell mass and *FFM* by external counting of ^{40}K. In humans the potassium content of *FFM* is reasonably constant. Chemical analysis of a limited number of human cadavers yielded values of 2.66 and 2.50 g potassium/kg *FFM* in males and females, respectively.[89] Less direct approaches with whole body counting and determination of *TBW* or densitometry yielded similar results.[90, 91] The errors of the method originate from the natural background radiation, difficulties to discriminate the ^{40}K gamma ray from other radiations and from the assumed ratio of potassium to *FFM*.

Computer tomography (CT) and magnetic resonance imaging (MRI)

CT can be used to estimate the amount of total or regional adipose tissue, and the values are highly correlated with body fat estimated by ^{40}K counting or densitometry.[92-94] MRI can be obtained without known health risks, and is therefore suitable for determination of adipose tissue. MRI can discriminate between visceral and subcutaneous adipose tissue accumulation,[95] and has even been used to assess *TBW* in animals.[96] Both CT and MRI are the methods of choice for measurement of visceral adipose tissue. The relationship between body fat and total adipose tissue is not resolved, and CT radiation and the high equipment costs limits the clinical usefulness.

In vivo neutron activation analysis (IVNAA)

IVNAA is a technique used to measure major body elements such as nitrogen, hydrogen and chlorine directly, as opposed to volumes and attenuation by other nuclear-based methods. When a subject is scanned through a neutron beam, some nuclei become excited for a short time and then return to their lower ground state with the emission of gamma rays that can be collected to form a composite spectra.[97] The method has been validated against wet chemical analysis of two cadavers[26] and phantoms.[98] In vivo precision is 5% for total body nitrogen and total body chloride. Together with $^{3}H_2O$ dilution a five-compartment model including water, protein, minerals, carbohydrate and fat has been developed, and used in ICU patients.[99, 100] Inaccurate estimation of *TBW*, the validity of assumptions regarding the ratio between different compounds, random errors, positioning of the patient, variations in body habitus and in the measurements of body habitus constitute some of the potential sources of error for the IVNAA method. The high instrument cost limits the availability of the method.

Near-infrared interactance (NII)

NII is a technique based on the principles of light absorption and reflection using near-infrared spectroscopy. It is performed by scanning over near-infrared wavelengths of 700-1100 nm. The instrument is suitable for field studies of body fat in non-obese subjects,[101] but has not yet reached a precision that could challenge DXA.[102]

Table 3
Transformation of units for ethanol

Measured unit	Transformed unit
1.00 g/L	21.7 mmol/L
1.00 mmol/L (mM)	0.0461 g/L
1.00 vol% (vol/vol) in water	0.792 weight% in water
1.00 weight% (w/w) in water	1.26 vol% in water
10.0 weight% (w/w) in water	12.3 vol% in water
1.00 g/L whole blood, *B-Hb* 140	1.19 g/L of the water fraction of the blood
1.00 g/L whole blood	0.947 g/kg whole blood
1.00 g/L whole blood	1.12 g/L plasma
1.00 g/2300L expired air	1.00 g/L venous whole blood

Bioanalytical and sampling issues of ethanol

Units and transformations

It is easy to become confused by the many different units used to report ethanol concentrations in alcoholic beverages and laboratory samples of blood, serum, urine and breath (Table 3). Ethanol (C_2H_5OH) has a molecular weight of 46.1 Daltons, which means that g/L can be transformed to mmol/L or mM (mg/L/46.1 = mmol/L). The density of ethanol is 0.792 kg/L (specific gravity at 25°C), which makes it possible to transform units of weight/weight to volume/volume if the density of the solvent is also known. Density is temperature dependent and at 15°C ethanol has a density of 0.798 kg/L. Note that the transformation of vol/vol to w/w and the reverse is dependent on the prevailing ethanol concentration, for example, 100 vol% is the same as 100 weight% but the value in Table 3 is applicable for all ethanol data in human experiments (1.262 for 1.0 weight%, 1.266 for 0.01 weight%). For water the density 1.0 is used for all practical purposes, but if the solvent is whole blood the density of whole blood must be used (1.055).

The blood:breath ratio of ethanol

The partition coefficient of ethanol between blood and air has been determined in-vitro as 1756:1 at 37°C.[103] The equilibration is strongly temperature dependent and the partition coefficient increases by ±6.5% per degree centigrade.[103] A physiological relationship exists between the concentration of ethanol in blood and breath, and breath-alcohol testing instruments are widely used in forensic casework instead of taking blood samples. Indeed, the breath-analysers used are usually calibrated by use of an *in-vivo* blood:breath factor to give results in close agreement with venous blood alcohol concentration. A common value for the *in-vivo* blood:breath ratio in the post-absorptive state of alcohol metabolism is 2300:1,[104-107] although for legal purposes, a ratio of 2100:1 is generally used.[108] A considerable variability in the blood:breath ratio of ethanol has been noted in recent studies with modern technology, with CVs of 9-19%.[108, 109] Among other things, the value appears to be higher after reaching C_{max} than during the ascending phase of the concentration-time plot.[110-114] In a recent review on the blood:breath alcohol ratio[115] it was suggested that a constant exhaled ethanol

concentration is never reached during a single prolonged exhalation because of interactions of ethanol molecules with the mucous surfaces in the conductive airways via diffusion from the bronchial circulation rather than with alveolar air.[116] Several sources of error need to be considered when breath-alcohol measurements are used instead of blood samples such as lung disease, vital capacity, forced expiratory volume, faulty heating of exhaled air, changed body temperature in the subject, and errors in instrument calibration.

Chemical properties, lipophilicity and endogenous levels of ethanol

All primary alcohols have a similar structure with one hydroxyl group (-OH), attached to a saturated carbon chain. Ethanol, CH_3-CH_2-OH, is formed during fermentation of grapes, or other parts of plants that are high in sugar or starch, together with water and yeast. When the concentration of ethanol reaches about 15% (vol/vol) most yeasts die and fermentation terminates. Higher ethanol concentrations can be reached by a distillation process. Ethanol provides 7.1 kcal/g (29 kJ/g) when oxidised to carbon dioxide and water in the body.

The lipid:water partition coefficient of ethanol (P) varies considerably depending on the nature of the lipid and aqueous phase studied. Whereas P for *octanol*:*water* is close to 0.5,[117, 118] P for *oil*:*water* is 0.035[117] and resembles the value of 0.09 obtained for P *membrane*:*buffer*[119] when calculated from other solvent systems. Ethanol has an infinite solubility in water and low solubility in lipids, which means that only a small fraction of the amount ingested will dissolve into body fat. If a normal subject has 40 L body water, 20 kg body fat and a lipid:water partition coefficient of 0.05, then theoretically 2.5% of a given ethanol dose could disperse into the body fat and the *TBW* would be overestimated by the same figure. A similar relationship can be obtained from the ethanol partition coefficients between *oil*:*air* and *blood*:*air*.[120]

Trace amounts of endogenous ethanol are produced in the gastrointestinal tract from micro-organisms such as yeasts or bacteria acting on carbohydrates from the diet. The concentration of endogenous ethanol is in the range 0 - 0.0008 g/L in whole blood and lacks clinical or forensic relevance.[121] These very low concentrations have been determined by gas chromatography, and the limit of detection could be lowered to about 0.0001 g/L by a salting-out technique. This entails saturating the biological specimen with an inorganic salt (NaCl or Na_2CO_3) prior to equilibrium at 50 or 60 degrees C. The added salt helps to increases the vapour pressure of non-electrolytes, such as ethanol, relative to water thus boosting the sensitivity of the analysis. Endogenous concentrations of ethanol can be increased in rare instances of severe infections with *candida albicans* in Japanese subjects or in bacterial peritonitis.[122, 123]

Ethanol pharmacokinetics

Reliable methods for analysing ethanol in small volumes of blood were developed in the 1920s.[124] Widmark presented the pseudolinear concentration-time curve for ethanol elimination in the 1930s.[125] The enzyme mainly involved in the oxidation of ethanol is hepatic alcohol dehydrogenase (ADH), which was first isolated from horse liver in 1948.[126] A few years later the wet chemical oxidation method for blood-alcohol analysis was replaced by an enzymatic oxidation, the ADH-method, having better sensitivity and specificity for determination of ethanol.[127] The limit of quantitation of ethanol was now lowered from 0.1 to 0.01 g/L in body fluids, which allowed Lundquist and Wolthers[128] to show that the entire post

absorptive ethanol concentration-time curve took the shape of a hockey stick. They also showed that concentration-time data could be fitted to a kinetic model with Michaelis-Menten elimination. Gas chromatography is now the method of choice for ethanol analysis in research and forensic science and a high accuracy and precision is possible. For a single determination the coefficient of variation is 1.0% at a blood-ethanol concentration of 0.8 g/L.[129]

Absorption

Absorption of ethanol across biological membranes occurs by passive diffusion along concentration gradients in the same transmembrane channels that allow the passage of water.[104] Absorption from the stomach begins immediately after the start of drinking an alcoholic beverage. Because the surface area for absorption is considerably larger in the small intestine than in the stomach, available data suggests that the absorption rate is about 30 times faster from the duodenum and jejunum than from the stomach.[130, 131] Therefore, the rate of gastric emptying seems to be the most important factor influencing the rate of absorption after oral intake of ethanol. Several factors that modulate gastric emptying have an impact on the absorption rate of ethanol, such as the presence of food in the stomach before drinking starts,[132-134] cigarette smoking,[135] drugs that retard or accelerate gastric emptying,[136-139] the concentration of alcohol in the drink,[131] and thus choice of alcoholic beverage.[140] The feeding state has major importance for within- and between-subject variation in the pharmacokinetic parameters of absorption.[132] The non-linear kinetics of gastric emptying rate[141, 142] makes it difficult to apply a pharmacokinetic model to the absorption part of the ethanol curve.[143, 144] Another factor that has been almost completely ignored by most investigators is the blood flow rate in the gastrointestinal mucosa, which might have a major influence on rate of absorption of ethanol especially after small doses are ingested.[145, 146]

First-pass metabolism (FPM) refers to the additional fraction of a dose of ethanol that is metabolised in the liver or gastrointestinal tract before ethanol is diluted in the total body water.[147] The significance of FPM has been much debated in recent years particularly whether this occurs in the liver the gastric mucosa or in both places.[147-152] Some investigators maintain that the stomach is the main site of FPM,[153-155] whereas others argue that it is the delivery rate of ethanol to the liver that governs the amount of FPM and that the concentration of ethanol in portal venous blood is of crucial importance.[147, 156] This means that the FPM of ethanol is closely related to the ethanol absorption and thereby also to gastric emptying rate. Furthermore, the Michaelis-Menten kinetics of ethanol metabolism invalidates the method of comparing areas under the concentration-time curve (*AUC*) after oral and intravenous dosing to assess bioavailability of the drug.[157] Even the definition of FPM has been debated.[147, 158]

In summary the presence of food in the stomach at the time drinking begins will delay gastric emptying resulting in slower absorption and a lower peak blood concentration and also diminished impairment of the ability to drive a motor vehicle. Gastric emptying has a major impact on within- and between-subject variation in C_{max} and apparent first-pass metabolism.

Distribution

Ethanol is believed to disperse into the total body water.[104, 159] The fat free fraction of body mass is not only related to the size of the subject but also to obesity, gender and age. As *TBW* is closely related to *FFM*, these factors are important determinants of the volume of distribution of ethanol and thus also organ toxicity and overall risk of ethanol exposure.[160-162] In epidemiological studies of medical disorders associated with heavy drinking the notion of

reporting total dose ingested has been questioned. Instead, dose/kg or dose/kg body water seems more appropriate when subjects of both sexes are included.[163-165] A number of investigators have used ethanol as an index of *TBW*, and this topic will be covered in greater detail later.

The distribution rate of ethanol into body fluids is obscured by the rate of absorption. In many drinking experiments this confounding influence has not been well recognised. At the start of the 1970s ethanol was investigated as an anaesthetic agent for minor gynaecological surgery and the distribution phase of ethanol was demonstrated after intravenous administration.[166, 167] Arterio-venous equilibrium occurred within 7 minutes. The utility of ethanol as an anaesthetic agent was abandoned because of unacceptable nausea and hangovers.[168] Later, a two-compartment model was applied to data after intravenous infusion of ethanol and the apparent half-life of the distribution phase was found to be 9.5 min.[169]

Metabolism and Excretion

Extent of metabolism

About 95% of an absorbed dose of ethanol is eliminated by metabolism in the liver and another 1% is conjugated with glucuronic acid and excreted in urine. Losses into the breath are proportional to the amount of ethanol in blood, but as natural losses of water in the breath are only about 30 mL per hour, this route of elimination is less than 1% in most cases. Loss of ethanol with the faeces, sweat or by transdermal convection is also negligible under normal conditions. The urinary ethanol concentrations are proportional to those in the blood and excretion might become significant if diuresis occurs at high blood alcohol levels. Normally however, excretion of ethanol in urine is less than 5% of the dose ingested.

Metabolic pathways

Three different enzymatic pathways regulate the metabolism of ethanol (Figure 1). Alcohol metabolising enzymes are distributed throughout the body, but at least 90% of the metabolism takes place in the liver.

Alcohol dehydrogenase (ADH) is the most important enzyme involved in oxidative degradation of ethanol. Six classes of ADH enzymes have been identified and some of them are polymorphic with two or more alleles. They show a wide variety of V_{max} and K_m values *in vitro*, and these can be incorporated into a physiologically based pharmacokinetic (PBPK) simulation,[170] but it is almost impossible to obtain sufficient *in vivo* data for such calculations. Class IV isozymes of ADH are predominantly located in the gastric mucosa, and much of the discussion regarding FPM focuses on whether the metabolic capacity of the stomach is sufficient to account for FPM, considering the weight of the gastric mucosa is only ~70 g[156] compared with 1500 g for the liver. The high K_m of class IV gastric ADH together with a very high ethanol concentration in the alcoholic beverage ingested speaks in favour of a significant FPM.[153, 155] However, this notion has been challenged by calculations based on measured gastric absorption rate.[156] The ADH isozymes of the liver appear in different proportions for Caucasians, African Americans and Asians especially Japanese.[171]

The rate of the ADH-enzymatic oxidation depends on alcohol delivery rate, the re-oxidation of NADH to NAD^+, and the activity of acetaldehyde dehydrogenase (ALDH).[172, 173]

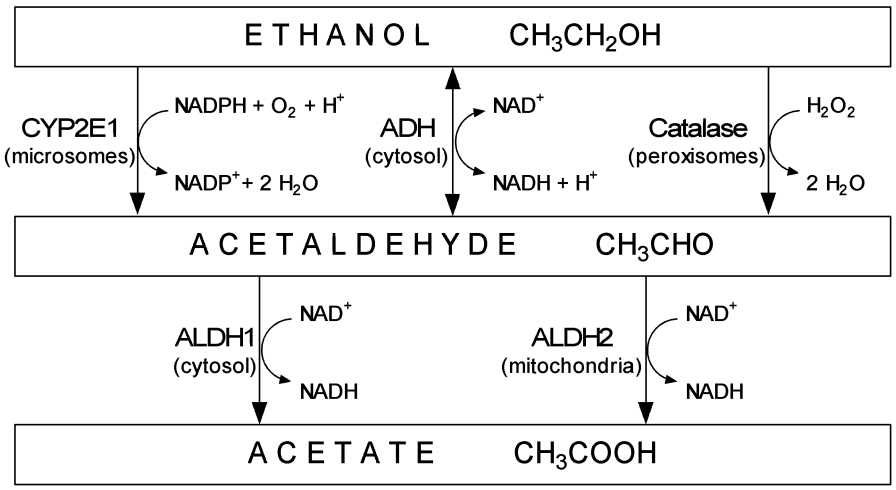

Figure 1
Metabolic pathways for ethanol. Alcohol dehydrogenase (ADH) is quantitatively most important at low alcohol levels, and Cytochrome P450 CYP2E1 is the inducible pathway.

Thermodynamically the reduction of acetaldehyde to ethanol is favoured at physiological pH and temperature but the very low K_m for ALDH means that acetaldehyde is very effectively oxidised. The concentration of free acetaldehyde in hepatic venous blood is more than 1000 times less than the ethanol concentration. In certain Asian populations the inactive ALDH2*2 allele is predominant making these individuals sensitive to even small amounts of alcohol owing to higher concentrations of acetaldehyde being present in the blood.[174] Homozygotes are almost completely protected against the development of alcohol dependence, and experience the effects of acetaldehyde including the alcohol-flush reaction even after very small amounts of ethanol are taken.[175]

Several models have been proposed to explain the hepatic metabolism of ethanol with Michaelis-Menten elimination kinetics.[176, 177] Because ethanol is rarely measured in the portal and liver veins, most investigators apply data from peripheral venous blood concentrations to a well-stirred model.[147] However the determination of K_m is extremely sensitive to the choice of model, the true hepatic concentration of ethanol and the hepatic blood flow rate[178, 179], which makes the scaling from *in vitro* estimations to *in vivo* predictions very tricky. The parallel tube model has occasionally been applied to liver kinetics of ethanol.[178, 180]

The microsomal ethanol oxidizing system (MEOS), located in the endoplasmic reticulum of hepatocytes, contains the cytochrome-P 450 isozymes, which are a group of important enzymes for oxidative metabolism of xenobiotics, prescription drugs and endogenous substances. The most important component for ethanol metabolism is the inducible CYP2E1 isozyme.[181, 182] Induction is achieved by substrate stabilisation of the enzyme, which decreases the turnover rate of enzyme.[183] It has been suggested that most of the observed increase in rate of ethanol metabolism associated with chronic heavy drinking is accounted for by induced CYP2E1 enzyme. Blocking CYP2E1 by drugs has shown that CYP1A2 is also capable of ethanol metabolism,[184] but this has so far not been characterized in humans. The MEOS system

requires oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to oxidise alcohol. A K_m exceeding 0.50 g/L has been reported *in vitro*.[172] Efforts to model two parallel Michaelis-Menten systems have been provided in rabbits[185] and humans.[186]

Catalase is found in liver peroxisomes. However, this pathway probably contributes little to the overall elimination of ethanol *in vivo*. Catalase activity is limited by the availability of the toxic co-substrate hydrogen peroxide that hardly exists in sufficient amounts in the liver to play any quantitative role.

Factors affecting the rate of elimination

The elimination rate of ethanol is often presented as a single value depicting the descending phase or slope of the pseudolinear part of the post-absorptive blood-alcohol concentration-time plot, k_0, or as the amount of ethanol cleared from the body per hour, the alcohol elimination rate, *AER*. A common value for k_0 is 0.15 g/kg/h (range 0.10-0.24) and *AER* is about 0.10 g/kg/h or 7 g/h for a 70 kg subject.[187] Most experiments on ethanol kinetics have been performed under controlled drinking conditions after a standardized dose is ingested in a short time (5-15 min) either in the fasting state or 1 hour after eating a meal. Even if this is unrealistic compared to social drinking, a host of factors have been identified that impact on the elimination rate of ethanol such as gender,[188, 189] age,[190, 191] ethnicity,[192-194] circadian rhythm,[195, 196] and regular high alcohol intake in alcoholics.[187, 197] The size of the liver itself relates to the ethanol elimination rate in rats,[198] and seems to explain some of the ethnic or gender differences in humans.[193, 199] Liver blood flow is likely to be important after small doses are ingested because the intrinsic clearance of ethanol in the liver is high which makes the rate of ethanol elimination dependent on blood flow. The model parameters of ethanol elimination are often confounded by the rate and extent of absorption seen in many controlled drinking experiments. Furthermore, concurrent distribution is rarely considered, and the multiple enzymatic pathways of ethanol metabolism must be simplified as a single enzyme system.

A better alternative to the zero-order elimination rate k_0 is the Michaelis-Menten elimination kinetics characterized by the maximal metabolic rate V_{max} and the blood concentration that corresponds to half of that rate, K_m. These kinetic parameters are not easy to determine from controlled dosing studies when alcohol is given orally.

Ethanol dilution as a measure of total body water

To predict blood ethanol concentration Widmark derived the following equation:[125]

$$a = c_0 \cdot p \cdot r \qquad (1)$$

where a = ethanol dose (g), c_0 = the blood ethanol concentration that would result if all the ethanol was immediately distributed into its total volume of distribution (g/kg blood), p = body weight (kg) and r = ethanol concentration ratio between the total body and blood, the so-called Widmark factor. Compartmental pharmacokinetic models were developed much later although today one might say that the Widmark equation represents an one-compartment model with zero-order elimination kinetics. When Grüner drew the conclusion that ethanol is dissolved exclusively in the body water[65] he realised that both c_0 and r depended on the *TBW* of each

particular subject. The concentration ratio r_w between the water concentration in the body *TBW* (w/w) and the water concentration in the blood fB_{water} (w/w) is

$$r_w = \frac{TBW(w/w)}{fB_{water}(w/w)} \tag{2}$$

With $r = r_w$ equations (1) and (2) can combine to form

$$TBW(w/w) = \frac{a \cdot fB_{water}(w/w)}{p \cdot c_0} \tag{3}$$

This equation can be modified to express *TBW* directly in litres. The amount of alcohol in the body has been exchanged with the dose D_{po} or D_{iv} for oral and intravenous administration, respectively.

$$TBW = \frac{D_{iv} \cdot fB_{water}(vol/vol)}{C_0} \tag{4}$$

The above formula is almost identical to the general dilution formula, namely volume = dose/concentration at equilibrium. It is important to note that Widmark reported c_0 in units of mass/mass. Today the method of gas chromatography is the gold standard for analysis of blood ethanol and results are reported as mass/volume units. This is denoted C or C_0 in the particular case of back extrapolation to time zero. The measurement units for fB_{water} are mass/mass, which is confirmed by the figure 80% used by Grüner.[65] Therefore, either C_0 must be transformed to concentration of ethanol in the water fraction of the blood or fB_{water} needs to be reported as volume/volume units. The transformation must be done with the aid of the blood density expressed as mass/volume (1.055).

After the pioneer work by Grüner[65] there was a limited interest in measuring *TBW* by the ethanol dilution method and only a few papers appeared over the following years.[200-202] A renewed interest in the method of ethanol dilution appeared recently with the introduction of quantitative breath-alcohol analysers that furnish a *non-invasive* way to monitor blood-ethanol and to derive *TBW* in a clinical setting.[203, 204] Various methods for measuring *TBW* in humans by ethanol dilution have been used (Table 4).

Two articles described use of urine samples for *TBW* measurements.[205, 206] Although these have the benefit of non-invasive sampling, possible shortcomings include pooling of urine in the bladder that makes the time point of the sample analysed uncertain if urinary excretion is variable, the presence of residual urine, diuresis induced by ethanol and the difference between a density of 1.000 and the actual density of urine.

Allowing the volunteer subjects or patients to drink the required dose of ethanol is non-invasive, but introduces a number of sources of error such as incomplete absorption, first-pass metabolism in the liver or gastric mucosa, and a difficulty to define zero-time if there is a lag time before ethanol reaches the portal vein and liver where pseudo zero-order elimination occurs. Moreover, the subjects must be in the fasting state before drinking otherwise the bioavailability of ethanol will be reduced thus introducing a significant error in the calculation of *TBW*. Only data from the post absorptive part of the concentration-time curve should be used owing to the complicated patterns of gastric emptying.

Table 4
Measurement of TBW in humans by ethanol dilution, mean ± SD.

Subjects	*bw (kg)*	*TBW (L/kg bw)*			Model and methods [1]	Reference
Gender (M, F), n		*ethanol*	*other*	*relation*		
M 27, volunteers	70	61	-		1-comp, 0-order, *VBAC* photometry, po 0.5 g/kg *bw*	Grüner 1957 [65]
M 10, volunteers	71.8 ± 8.7	52.6 ± 5.5	54.9 ± 5.7 Antipyrine	0.959 ± 0.023		
M 10, volunteers	-	53.0 ± 3.1	53.8 ± 3.7 Antipyrine	0.959 ± 0.023	1-comp, 0-order, *VBAC* photometry, po 0.5 g/kg *bw*	Grüner 1961 [207]
?			no		1-comp, 0-order, *UAC*, 0.35g/kg *bw*	Pawan 1963 [205]
M 35, healthy		60.3 ± 6.0	61.8 ± 5.0 ^3H_2O	0.977 ± 0.073	1-comp, 0-order, *BrAC*, GC, po 0.5-0.35 g/kg *bw*	Loeppky 1977 [200]
M 18, F 10 HD-pat	67.4	57.9 ± 8.1	no		1-comp, 0-order, *VSAC* GCx2 + ADHx3, po 0.5 g/kg *bw*	Walle 1980 [201]
M 12, healthy	59.3 ± 9.8	59.1 ± 3.1	no		1-comp, 0-order, po 0.6g/kg *bw* *UAC*	Avadhany 1986 [206]
M 15	80.5 ± 15.4	40.9 ± 8.5 40.3 ± 8.6	no		1-comp, 0-order, po 0.6g/kg *bw*, plasma and blood	Jones 1992 [208]
M 15, healthy	73.9	58.2 ± 2.6	59.8 ± 3.0	0.978 ± 0.021	1-comp, 0-order, *VSAC* GCx2 + ADH, po 0.8 g/kg *bw*	Endres 1994 [203]
F 5	71.6	50.6 ± 2.2	51.1 ± 2.3 D_2O			
M 8, PD-pat	84.0 ± 10.8	53 ± 7	49 ± 4 Anthropo-metry	1.08	1-comp, 0-order, *BrAC* by dual beam IR, po 0.3g/kg *bw*	Dahl 1999 [204]
F 11, PD-pat	67.3 ± 18.2					
M 8, healthy	72.7 ± 9.5	51.7 ± 4.9	61.2 ± 4.6 D_2O	0.845 ± 0.045	2-comp, MM, *UAC*: GC, *BrAC*: IR, iv 0.4 g/kg *bw*	Norberg, paper V
		53.5 ± 5.4		0.873 ± 0.038	*VBAC*: GC	
F 8, healthy	70.1 ± 6.7	45.3 ± 4.7	53.8 ± 5.9	0.844 ± 0.045	*BrAC*	Norberg, paper V
F 6		45.9 ± 5.7	52.3 ± 6.2	0.879 ± 0.040	*VBAC*	

[1] Compartments (0-1-2), elimination (0-order or MM = Michaelis-Menten elimination), site of measurement (V=vein, Br=breath, B=blood, S=serum, U=urine, AC=alcohol concentration), method (GC=gas chromatography, IR=infrared, ADH=enzymatic), administration (po or iv), dose (g ethanol/kg *bw*).

Furthermore, distribution and absorption easily becomes confounded and are therefore often neglected. The precision of C_0 is also dependent on the number of concentration-time points on the descending phase and the length of the back extrapolation to the time of dosing (Figure 2). Too high a dose or too rapid administration can also be a problem because of inebriation and other side effects of ethanol impairment.

Loeppky *et al.*[200] investigated *TBW* in 35 healthy males with ethanol and ^3H_2O dilution. They found a good agreement between the two methods, r = 0.90, but the individual differences were considerable and exceeded 10% in 9 subjects. They also assessed *LBM* by hydrodensitometry and derived regressions between *LBM* and *TBW* by ethanol dilution.

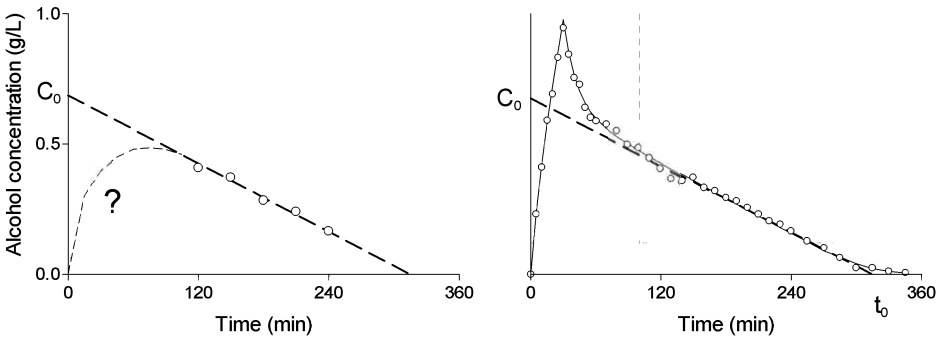

Figure 2
Left panel: Concentration-time plot after oral administration according to Grüner,[65] where C_0 is the extrapolated concentration at the start of drinking.
Right panel: Concentration-time profile after intravenous infusion of ethanol. Dotted lines mark the cut-off from the initial phase (100 min) and terminal (elimination) phase (0.12 g/L), respectively.

Endres and Grüner[203, 209] compared *TBW* assessed by D_2O dilution with the volume of distribution for ethanol and found a remarkably good correlation (r = 0.966). They used a relatively high dose of ethanol, 0.8 g/kg *bw*, that possibly overcame some of the problem with first-pass metabolism. Ethanol was measured by gas chromatography and enzymatically and transformed from promille (‰) ethanol in whole blood to ‰ ethanol in the water fraction of the blood by considering blood water content (w/w). The use of ‰ as unit of ethanol measurement creates some uncertainty whether this value is in g/kg or g/L, especially as they stated that a certain sample volume was used for analysis. This could give rise to an overestimation of the ethanol space by 5%.

Jones *et al.*[208] showed that *TBW* could be assessed from both blood and plasma concentrations of ethanol and that similar results were obtained. This agrees with reports that ethanol content of blood and plasma is proportional to the water content of the two specimens.[210-212] *TBW* obtained from extrapolation to C_0 of the ethanol concentration time profiles in blood and plasma was compared with *TBW* from anthropometric data.[76, 213] A mean difference of 2% was observed.

In one study the method of ethanol dilution was used to determine *TBW* in 37 anaesthetised dogs.[202] The correlation between the ethanol volume of distribution and *TBW* by ^3H_2O dilution was high, r = 0.967, although the intra-gastric ethanol space was thought to be overestimated owing to delayed gastrointestinal absorption. First-pass metabolism possibly contributed to this discrepancy. Volume changes resulting from acute saline infusions in anuric animals were reflected by changes in both the ethanol and ^3H_2O spaces. An ethanol dose of 0.35-0.5 g/kg *bw*, arterial plasma specimens, and a single compartment model with zero-order elimination was used.

In summary the reproducibility of the ethanol-dilution method in comparison with the isotope-dilution method for measurement of *TBW* has been poorly investigated and the results can be questioned because the experimental designs have often involved oral dosing of the tracer and the kinetic analysis of the ethanol concentration-time data has not been appropriate.

AIMS OF THE THESIS

The aims of this thesis can be summarized as follows:

- To investigate the properties of ethanol distribution and elimination and to develop appropriate pharmacokinetic models for intravenous ethanol, which is necessary to obtain correct estimates of the ethanol volume of distribution (paper II, III, IV).

- To find an optimal intravenous ethanol dose and infusion rate that provides sufficient kinetic data for modelling while avoiding untoward side effects of ethanol (paper II).

- To evaluate the impact of the feeding state on the rate of elimination for ethanol given by intravenous infusion (paper I).

- To estimate the influence of the local peripheral blood flow on blood ethanol concentrations and pharmacokinetic parameters of ethanol (paper III).

- To evaluate the impact of site of sampling and the magnitude of arterial-venous differences in ethanol concentration on pharmacokinetic parameters of ethanol (paper III, IV).

- To compare ethanol dilution with deuterium oxide dilution for estimating total body water in healthy volunteers (paper V).

ETHICAL CONSIDERATIONS

All experimental protocols used in this thesis were approved by the local ethics committee at Huddinge University Hospital. Factors to consider included the use of healthy paid volunteers without any alcohol problems, the use of indwelling catheters in a cubital vein and in one study an arterial line, volume load, volume of blood sampled and potential toxicity of ethanol and deuterium oxide. Informed consent was obtained from the volunteers before each study and they were given a physical examination and various biochemical tests were made to check liver function, circulatory and renal status.

The infused volume of ethanol and isotonic fluid was 4-8 mL/kg *bw* or 280-560 ml in a 70 kg subject. The effect of this volume loading in 15-60 minutes was considered negligible.

An *indwelling intravenous catheter* was inserted into a cubital vein of each subject and this procedure caused no discomfort apart from the pain of the initial penetration of the needle.

An *arterial line* (paper III) was inserted in the radial artery after local anaesthesia by an experienced anaesthetist. The complications of this invasive technique are well investigated.[214, 215] No adverse side-effects were observed.

The *volume of blood sampled* amounted to about 200 mL per subject in paper III and 200 mL per experiment in study IV. These volumes can be compared to 450 mL of whole blood normally taken by the hospital blood bank.

The *toxicity of alcohol* (all papers) is low and the dose of 0.4 g/kg *bw* equals 28 g in a 70 kg subject. This amount of alcohol is contained in about 2 glasses of wine (250 mL) or in 2½ cans of Swedish "folköl" (1.25 L of 2.8 vol%). The intravenous application gave a maximum blood-ethanol concentration of about 1.0 g/L (~1 promille) at the end of the infusion. This caused a moderate feeling of inebriation, but this sensation rapidly diminished as the ethanol distributed from the central compartment into the ethanol volume of distribution. One subject experienced severe inebriation by the shortest ethanol infusion (0.4 g/kg *bw* in 15 min, paper II), but did not vomit and recovered quickly. Volunteers were observed until breath-alcohol concentration had reached zero, and were warned about driving home because of possible hangover effects.

Deuterium oxide toxicity (paper V) has been estimated in animal experiments. A critical but non-toxic threshold of D_2O is about 10%, and the limit of absolute harmlessness is about 1-2%.[216] The dose used resulted in an atom per cent excess of less than 150 ppm or about a doubling of the background level. This equals a level of 0.03%, far from any risk of toxicity. The use of D_2O did not necessitate evaluation by the hospital isotopic committee.

MATERIAL AND METHODS

Subjects

All subjects who participated in the experiments described in this thesis were paid volunteers, mostly hospital employees, in good health. Demographic details for the 46 volunteers are summarized in Table 5. All subjects were accustomed to moderate drinking and 24 were smokers but they were not allowed to smoke during the experiments.

Study conditions

General study conditions

All studies were done in a clinical research laboratory setting at the Huddinge University Hospital. The subjects arrived in the morning after an overnight fast. A commercial ethanol solution 100 g/L (Apoteksbolaget, Umeå, Sweden) with glucose 50 g/L was used for intravenous infusion of ethanol in papers I, III and IV. In paper II ethanol 60 g/L in saline was used instead. Ethanol infusions were performed via an indwelling catheter in the cubital vein. The cannula was removed after the end of infusion. Specimens of whole blood were taken into Vacutainer™ tubes from an indwelling catheter in a forearm or cubital vein on the opposite arm. The catheter was kept patent by flushing with normal saline after each sampling. The subjects remained non-smoking and seated in a hospital bed in a semi-recumbent position, often watching video films or reading between sampling schedules.

The high osmolality of the ethanol infusions can sometimes cause discomfort or even pain during infusion. Therefore the subjects also received small amounts of an isotonic electrolyte solution (Ringer's acetate) in study I. In study II an ethanol solution with a lower concentration was used for infusion to minimise discomfort. The problem seems to be related to the blood-flow in the vessel of the infusion. In paper IV we gave an equal amount of normal saline in the same vein and at the same rate as the ethanol infusion.

An outline of the various studies is presented in Table 6. A total of 110 ethanol infusions were made. By sampling in more than one vascular segment or in the breath a total of 190 alcohol concentration-time plots were obtained. The total number of measurement points in each study and number of blood and urine specimens taken for analysis exceed 3500.

Table 5
Demographic details of the subjects, mean ± SD.

Subjects	N	Exp[1]	Ser[2]	Age *(years)*	Heigth *(cm)*	Weight *(kg)*	*BMI* *(kg/m^2)*
Male volunteers	26	52	116	33.0 ± 5.2	180.7 ± 6.3	76.7 ± 9.8	23.5 ± 3.2
Female volunteers	20	56	72	34.2 ± 9.0	166.5 ± 5.6	67.9 ± 8.7	24.6 ± 3.8
All volunteers	46	108	188	33.5 ± 7.1	174.5 ± 7.3	72.9 ± 10.2	23.9 ± 3.1
Significance [3]				n.s.	$p < 0.001$	$p < 0.01$	n.s.

[1] Exp is the total number of experimental session in the group.
[2] Ser is the total number of series in the group based on simultaneous measurements at different sites such as breath and venous blood.
[3] n.s. is not significant.

Table 6
Study conditions

Paper	Number *n*	Repeats *r*	M/F [1]	Dose *(g/kg bw)*	Exp. time *(min)*	Site of sampling [1]	Inf. time *(min)*
I	12	2	6/6	0.4	180	V	45
II	6	5	0/6	0.4, 0.6	180	V	15, 30, 45, 60
III	12	2	12/0	0.4	210 +	A, V, Br	30
IV and V	16	2	8/8	0.4	360	V, Br	30

[1] V = venous blood, A = arterial blood, Br = exhaled breath, M/F = male/female

Paper I

Six female and six male subjects were studied in the fasted and fed state. On the same study day, two alcohol infusions were given. The subjects were randomly allocated to a light breakfast or hospital kitchen lunch. The lunch was served about 4 hours after the start of the first infusion. When a breath-alcohol analyser (Alcolmeter S-D2, Lion Laboratories Ltd., Barry, U.K.) showed negligible concentrations of ethanol after about 5 hours the second ethanol infusion was given. Inebriation was reported on a visual analogue scale. Alcohol was analysed in venous blood sampled at exactly timed intervals. The pharmacokinetic calculations of clearance, mean residence time and volume of distribution at steady state (V_{ss}) were performed by non-compartmental methods on individual data sets. The single compartment zero-order model was used to calculate the ethanol elimination rate from the blood.

Paper II

The rate of ethanol distribution into *TBW* was studied in six females who received infusions of the same ethanol dose (0.4 g/kg *bw*) in 15, 30, 45 or 60 minutes on four separate occasions. The infusions were given in random order and at least two days apart in each subject. The higher dose 0.6 g/kg *bw* was given in a second series of experiments. The degree of subjective alcohol intoxication was reported on a visual analogue scale. Alcohol was analysed in venous blood at exactly timed intervals. The ethanol concentration-time plots were analysed from the time of ending the infusion by a kinetic model where ethanol is distributed within a well-stirred single compartment and eliminated by a zero-order process (saturated Michaelis-Menten kinetics).

Paper III

Twelve healthy male volunteers participated in experiments where the influences of cooling and warming of the hand used as blood sampling site was studied. The impact of vasoconstriction or vasodilatation on ethanol concentrations and pharmacokinetics was also investigated. Each subject was given two ethanol infusions on the same study day. One infusion was a control experiment, and in the other experiment the sampling hand was either warmed or cooled. The experiments were performed in random order and a hospital lunch was served between the two experiments. Ethanol was measured in arterial and venous blood and in expired breath at exactly timed intervals. Pharmacokinetic analysis of the whole concentration-time data set was performed by a model with first-order distribution within a single compartment and followed by zero-order elimination.

Paper IV and V

Eight female and eight male volunteers received two ethanol infusions in the fasted state two days apart. Deuterium oxide was given as an intravenous injection immediately before the start of the ethanol infusion. Ethanol was measured in expired breath, venous blood and urine. Breath and blood samples were taken every 5 minutes for 4 hours and then every 15 minutes for the remainder of the study. Urine was obtained by voiding every hour or when necessary. Deuterium oxide was measured in blood serum, saliva and urine every hour. Pharmacokinetics was analysed by a two-compartment model with parallel Michaelis-Menten and first-order renal elimination applied to ethanol data from blood and urine or breath and urine. The obtained pharmacokinetic parameters were compared by analysis of variance (ANOVA) to establish the precision and sources of variability of parameter estimates. Ethanol V_{ss} was compared with total body water obtained by deuterium oxide dilution.

Methods of measurement

Blood alcohol by gas chromatography **(I, II, III, IV, V)**

Samples of arterial (III) or venous blood were taken from indwelling catheters into 5 mL Vacutainer™ tubes containing 20 mg NaF and 143 units of heparin (I, II, III), or into 3 mL tubes containing 45 units of lithium heparin (IV). Aliquots of whole blood, 100 µL, were removed from the tubes and diluted 11 times with n-propanol 80 mg/L as an internal standard. The blood with internal standard was transferred into 22 mL headspace sampling vials, which were immediately made air-tight with rubber-stoppers and crimped-on aluminium caps. A glass column packed with Carbowax C (0.2% Carbowax 1500 on Carbopack 80-100 mesh) was used as the stationary phase for gas chromatography. The analytical precision of a single measurement increased with the alcohol concentration. At 0.8 g/L the CV was less than 1.0%, but at 0.1 g/L CV was about 5%. The limit of quantitation was approximately 0.01 g/L.[129]

Urine alcohol by gas chromatography **(IV, V)**

The total volume of urine was measured at each sampling time, and a 5 mL aliquot was taken into an empty urine tube. The analysis of alcohol in urine was performed in exactly the same way as for blood.

Breath alcohol by infrared technique **(III, IV, V)**

Many volatile compounds can be analysed from their absorption of radiation at discrete wavelengths. Ethanol absorbs infrared radiation at several distinct wavelengths but for quantitative analysis the 3.4 micron range is used, which corresponds to the C-H stretching frequency in the molecule. To avoid interferences from other endogenous volatile substances containing C-H bonds, such as acetone, the absorption of IR radiation is monitored at two distinct wavelengths of 3.38 and 3.42 microns and the results are compared.

We used a DataMaster (National Patent Analytical Systems Inc., Mansfield, Ohio, USA) for breath-alcohol analysis. This is a quantitative infrared analyser where the subject provides a prolonged exhalation into an electrically heated plastic tube. The heating prevents condensation of water vapour on the walls of the tubing. According to the manufacturer the CV for a single determination of breath-alcohol is 3%. The subjects were asked to exhale for as long as possible to obtain end-expired alveolar air. A convenient exhalation time was tested

before the start of the experiments, and the subject was then asked to try to exhale in a similar way and for a similar manner during each measurement. We either used a single measurement mode (III) or duplicates (IV). The sampling chamber for infrared analysis was rinsed before, after and between the two samples with room air and duplicate analysis could be made within about 60 sec. With duplicates the CV was about 1% at an ethanol concentration of 0.4 g/L, which is better than that claimed by the manufacturers.

Inebriation assessed by visual analogue scale **(I, II)**

To evaluate the subjective degree of intoxication as a function of blood alcohol concentrations, the volunteers were asked to report their "feeling of inebriation" on a visual analogue scale (VAS) graded from 0 to 10. "Maximal imaginable feeling of inebriation" was indicated by 10. The concept of acute tolerance ("Mellanby Effect") was based on observations of greater behavioural impairment while ethanol levels were rising compared to when they were falling to the same blood ethanol concentrations in humans and dogs.[217, 218]

Blood water by desiccation **(IV, V)**

To account for changes in blood water concentration between the volunteers and within each experiment, 1 mL aliquots of whole blood were desiccated by heating to 100 °C for 24 h. The water content of the blood (w/w) was then estimated from the weight difference before and after heating.

Deuterium by isotope-ratio mass spectrometer **(V)**

We used the plateau method, where the subjects remain fasting throughout the equilibration period. We also made the measurement period long to make sure that complete mixing was obtained. Deuterium was given as an intravenous injection of 10 mL with 50% atom percent excess, which means that the subjects received about 5 mL of D_2O. The exact amount of D_2O was determined by weighing the syringe before and after injection on a precision scale. The dose was chosen to create an isotope excess of about 120-140 ppm in body fluids. The background level, about 100 ppm, was assessed in samples of blood, urine and saliva taken before the D_2O injection. Specimens for D_2O-analysis were taken after 15, 30 and 60 minutes and then hourly until 8 h. The subjects were encouraged to void every hour. Specimens were taken from urine, venous blood and in 10 subjects also from saliva. The blood specimens were centrifuged at 10°C, and serum transferred to 3.5 mL glass vials (Labora, Sollentuna, Sweden) that were immediately made air-tight by crimped-on aluminium caps and stored at 4°C until analysis within 10 days.

Isotope ratio enrichments of the samples were analysed in duplicate relative to laboratory reference water samples, standardized against the Vienna Standard Mean Ocean Water and Standard Light Arctic Precipitation, using a isotope-ratio mass spectrometer (SIRA, Series II, VG Isogas Ltd., Middlewich, Chesire, UK). Serum samples were distilled prior to analysis. The $^2H/^1H$ ratio of the specimens were analysed after reduction of water to hydrogen gas at 500 °C with the use of 200 mg zinc reagent (The Biochemical Lab, Indiana University, IN, USA). The analytical precision, including the preparation of analyte gas, was <1 ppm corresponding to a CV of 0.75%.

After complete equilibration in all body fluids, the $^2H/^1H$ ratio should be constant if the disappearance rate of both isotopes can be considered equal and no food or water is administered. *TBW* can be calculated from the $^2H/^1H$ ratio. The ratio of the deuterium dilution

space to *TBW* was assumed to be 1.041:1, which accounted for the nonaqueous hydrogen exchange.[16] Equilibration of D_2O was assumed to be complete after at least after 8 h. Therefore, samples were compared from 8 h and backwards until 2 samples were found to be within 2% of each other. The mean of all samples within ± 2% of the value for the last point in time was used for the calculation of *TBW*.

Cooling and arterialization of venous blood **(III)**

Arterialization of venous blood was performed by heating one hand in a special heating box with an electric heater and a fan. The hand was covered with cloth to prevent thermal radiation injury. The heating box method is a well-established way to obtain arterialized blood.[219-221]

Cooling was performed by putting the forearm in cold water, at about 15 °C. Cooling was started 15 min before the start of the ethanol infusion and maintained to the end of the experiment or at least for 3 hours. The temperature was chosen to avoid too much discomfort for the subjects. Cooling is sometimes used in pain research as a repeatable standardized pain stimulus.[222] Skin temperature was measured on the forearm close to the site of the blood sampling cannula and reached about 25°C after 60 min.

Transformation of ethanol in whole blood to ethanol in blood water **(IV, V)**

When ethanol dilution is used for determination of *TBW* it is the volume of distribution in the water phase of the body that is of interest. If whole blood is used for kinetic analysis one can use values from the literature as the fractional blood water content (fB_{water}). As for ethanol it is easy to get confused by weight% and volume% (see Table 3, page 19)

An improvement from using a single value from a textbook is to estimate the actual water content of the blood samples. The variation in water content between subjects arises mainly from differences in the erythrocyte fraction and plasma proteins. As we gave a rapid infusion and sampled about 200 mL of whole blood per session we caused a dilution of the blood during the experiment. To account for the variations between subjects and within experiments, we determined blood water at 0, 30, 90, 180 and 360 minutes. The values were transformed from weight/weight to vol/vol assuming a whole blood density of 1.055 at blood water 80% (w/w) and making a straight line to 1.0 at 100% (w/w). This yields the linear relationship

$$fB_{water}(vol/vol) = \left(1.275 - 0.275 \cdot fB_{water}(w/w)\right) \cdot fB_{water}(w/w) \qquad (5)$$

The density of whole blood and plasma has been reported as 1.056 kg/L and 1.027 kg/L, respectively.[223, 224] Density is also temperature dependent and often referred to the water density at 4°C. The density of plasma in healthy volunteers was 1.0310 at 4°C and 1.0205 at 37°C, respectively, and for whole blood 1.062 and 1.051, respectively.[225]

The values obtained for blood-water (vol/vol) were connected with straight lines and finally the whole blood ethanol concentration time data sets were transformed to ethanol in the water fraction of the blood by individual correction factors for each measurement point.

Pharmacokinetic models

One-compartment, zero-order elimination (I, V)

The classical Widmark model represents a single compartment with zero-order elimination, which means that a constant amount of ethanol is eliminated per unit time independently of the prevailing blood-alcohol concentration. Consequently, clearance increases with decreasing concentration of ethanol. This model is still much used in forensic science, because it gives a single figure for the blood ethanol elimination rate when only two specimens are available in forensic casework. Ethanol concentration can only be accurately described in the post-absorptive phase by the straight line

$$C = C_0 - k_0 \cdot t \qquad (6)$$

In this equation, C is the alcohol concentration in whole blood, C_0 is the concentration in whole blood if the ethanol dose had been immediately absorbed and dispersed in the entire volume of distribution (noted B in paper II and III), t is time after start of administration, and k_0 is the zero-order elimination rate, (noted β in paper II and III). Other notations for k_0 in the literature include β-slope or β_{60}[125, 188, 189, 226] BEDR for blood ethanol disappearance rate[227] or DLS for descending limb slope.[173]

Hybrid model with first-order distribution to an equilibrated single compartment and zero-order elimination (II, III)

The existence of an initial (assumed distribution) phase for ethanol became obvious after the testing of ethanol as an anaesthetic agent in the early 1970s.[167] The concept that ethanol distributes into the total body water for a limited time period and then stays completely equilibrated still persists among many investigators[104, 159] in spite of some theoretical shortcomings, that will be discussed later. Some researchers have mentioned or postulated multi-compartment pharmacokinetics of ethanol but still use a single compartment in their modelling.[179, 228]

When the concentration-time plot is extrapolated back to zero-time to obtain the y-intercept C_0 the uncertainty in this value depends on the coefficients in the regression equation relating $C(t)$ and sampling time. The smaller the residual standard deviation the higher the precision in estimating C_0. The proposed model aimed at decreasing the length of extrapolation to determine C_0, which theoretically increases the precision. It also helped to find the rate of distribution and thereby the time when the pseudo-linear part of the plot started. Alcohol concentration after the end of infusion could be described by

$$C = A_i \cdot e^{-\alpha \cdot t} + C_0 - k_0 \cdot t \qquad (7)$$

where A_i is the additional concentration of ethanol in blood at the end of infusion above the straight line of the Widmark model, and α is the first-order decay constant for the distribution. In study III the mathematical model was further developed to include all measurement points from the start of the ethanol infusion.

Two-compartment, parallel Michaelis-Menten and first-order renal elimination (IV, V)

The difficulty in fitting data to a combined linear and concentration dependent model can be overcome by use of non-linear regression software such as WinNonlin (Pharsight SCI, Cary,

NC, USA). The model contains six parameters and three differential equations. Clearance of ethanol was modelled as a capacity limited function of the concentration

$$CL = \frac{V_{\max}}{K_m + C} \tag{8}$$

where CL is the metabolic elimination of ethanol from the body (L/min). The maximal metabolic rate (V_{max}) and the concentration that gives half of that rate (K_m) are called the Michaelis-Menten constants. The turnover of ethanol in the central compartment then becomes

$$V_C \cdot \frac{dC}{dt} = \frac{D_{iv}}{T_{\inf}} - CL \cdot C - Cl_d \cdot C + Cl_d \cdot C_T - CL_R \cdot C \tag{9}$$

where V_C is the volume of the central compartment, Cl_d the intercompartmental distribution parameter, D_{iv} is the given amount of ethanol (g), T_{inf} is the infusion time, and the term D_{iv}/T_{inf} is set to zero for $t > T_{inf}$. In the peripheral compartment the equation is

$$V_T \cdot \frac{dC_T}{dt} = Cl_d \cdot C - Cl_d \cdot C_T \tag{10}$$

where V_T (L) is the volume and C_T (g/L) is the concentration of the tissue compartment, respectively. The accumulated amount of ethanol in urine A_e (g) can be calculated from

$$\frac{dA_e}{dt} = CL_R \cdot C \tag{11}$$

where CL_R is the first-order renal clearance of ethanol (L/min).

Model selection and validation

There are several ways to determine if one pharmacokinetic model is superior to another. Even if the F-test of variances shows statistical significance, this does not mean one model is necessarily better than the other. If the number of data points is abundant, significance may readily appear, but a parameter with a huge CV% is of limited value.

There are some practical rules like ”use the least complicated model that fits the data”. When compartmental models are compared, the most important difference arises between the one-compartment and multi-compartment models. The applied models are shown in Figure 8.

Weighting the data is often used to give a more even distribution of residuals, because the errors are usually more likely to be proportional to concentration of alcohol instead of being constant over the whole range of values. As a rule of thumb, weighting should be considered necessary when the values exceed a range of 20:1, but the most valuable information is obtained from the plot of residuals. To compare models with Akaike or Schwarz criteria or by F-test of variances, constant weighting of data are necessary, which means that weighting cannot be related to the predicted concentrations.[177, 229]

In general, non-compartmental analysis is not appropriate for dealing with non-linear kinetic systems. However, for comparing a robust parameter such as clearance, it might be useful to assess the influence of different study conditions.

Statistics

Conventional statistical methods were used to evaluate the results generated in this thesis and the software programs StatView™ (paper I-III) and STATISTICA® (paper IV and V) were adopted. Means, standard deviations (SD), standard errors of the mean (SEM, paper III) and coefficients of variation (CV) were calculated by normal methods. For skewed distributions medians and range were used (paper I and II). Partial F-test and the run's test were used to evaluate the model of paper II. Paired t-test or Wilcoxon's matched pair test were used to compare groups as appropriate. Differences between means were calculated together with 95% confidence interval (CI) of the mean difference (paper IV and V). Correlation-regression analysis was done by the method of least squares and association established by Pearson's correlation coefficient (*r*). Analysis of variance (ANOVA) was adopted to derive within- and between-subject components of variation for kinetic parameters, and repeated measures ANOVA as appropriate.

The issue of analytical precision has been repeatedly addressed in the literature.[230-232] Although the precision of a method, a pharmacokinetic model, or an experimental design can be assessed by repeated experiments, most studies on ethanol pharmacokinetics are performed using other experimental designs. Simultaneous assessment of between-day, between-subject and within-subject variance can be obtained by two-way ANOVA. The components of variance will differ according to the statistical model used. Another way to assess precision is to calculate the SD of the difference for a group of paired observations as proposed by Bland and Altman.[233] Interestingly, the variance obtained gives a slightly different value by this method and consequently a different precision compared with ANOVA. The main target of the criticism of Bland and Altman was the use of Pearson's correlation coefficient to assess the agreement between two methods. Correlation means association, which is not the same as agreement.

RESULTS

Paper I

Eating a meal increases the clearance of ethanol given by intravenous infusion.

Eating a meal increased the apparent clearance of ethanol from 0.24 ± 0.05 to 0.38 ± 0.03 L/min ($p<0.001$) and shortened mean residence time from 2.64 ± 0.57 to 1.63 ± 0.54 hours, when calculated by non-compartmental analysis. These parameters cannot be compared with similar parameters estimated from compartmental modelling. However, feeding also increased the zero-order elimination rate (k_0) from 0.132 ± 0.032 to 0.168 ± 0.032 g/L/min. Alcohol intoxication was less pronounced at the end of the ethanol infusions preceded by eating a meal ($p<0.01$). The variability in concentration-time profiles is displayed in Figure 3, showing that in the fed state peak concentrations are lower and the observed *AUC*s are smaller.

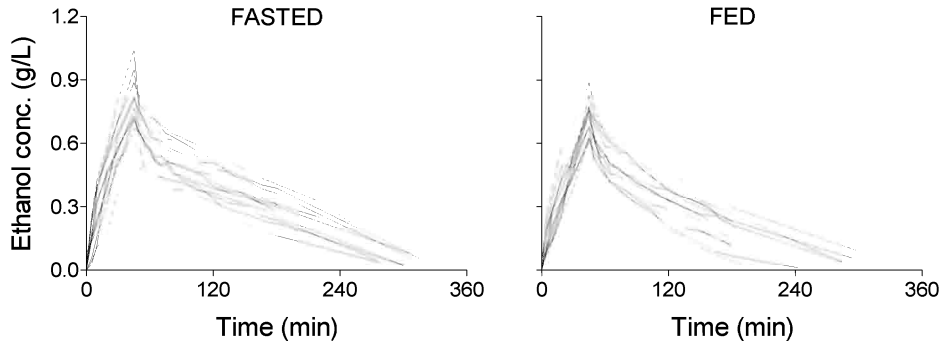

Figure 3
Spaghetti plot of data from paper I showing the individual variability and the effect of feeding state.

Paper II

Rate of distribution of ethanol into the total body water

The terminal data points followed zero-order elimination with a mean r^2 of 0.995. The exponential rate constant (α) was 0.118 ± 0.041 per minute corresponding to a distribution half-life of 6.6 ± 2.6 min. The ethanol infusion rate was associated with subjective inebriation ($p<0.001$), and the most rapid infusion rate caused pronounced inebriation in one subject. The "Mellanby effect" was confirmed by the subjective inebriation rating (Figure 4).[218]

Paper III

Pharmacokinetics of ethanol in arterial and venous blood and in end-expired breath during vasoconstriction and vasodilation

The curvefitting to the proposed model gave a mean r^2 of 0.992. The arterio-venous difference during infusion was doubled by cooling, whereas warming reduced the difference by 10-80%. The arterial-breath difference was positive during infusion, but always became negative within 5 minutes after cessation of the infusion.

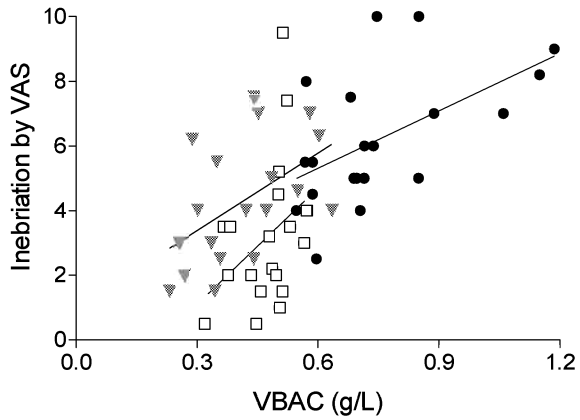

Figure 4
Alcohol inebriation assessed by visual analogue scale (VAS) vs. venous blood alcohol concentration (VBAC), after 50% of the dose (▼), r = 0.51, p = 0.026, at the end of infusion (●), r = 0.55, p = 0.013 and 30 minutes after the end of infusion (□), r = 0.38, p = 0.097. Data from paper II.

Cooling maintained the increased arterial-venous difference for another 45 min. The model parameter B (equivalent to C_0 and thus closely related to V_d) was 2% lower in venous blood compared with arterial blood, and this difference was increased to 10% by cooling the sampling hand. Breath sampling agreed better with arterial blood than with venous blood.

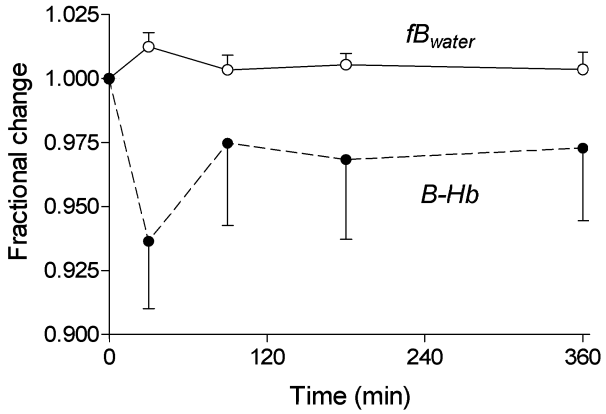

Figure 5
Changes in blood water fraction (fB_{water}), open circles, and haemoglobin (B-Hb), closed circles, as a function of time following a 30-min intravenous infusion of ethanol in 32 experiments (paper IV).

Paper IV

Within- and between-subject variation in pharmacokinetic parameters of ethanol by analysis of breath, venous blood and urine

The two-compartment model with parallel Michaelis-Menten and renal first-order elimination gave an excellent fit to the whole concentration-time profile after intravenous infusion of ethanol. The between-subject variability of ethanol concentration increased with the time after the end of the ethanol infusion. The mean ± total SD of the six model parameter estimates derived from breath and urine data were V_{max} 95 ± 21 mg/min, K_m 25 ± 20 mg/L, Cl_d 836 ± 149 mL/min, V_C 11.3 ± 2.0 L, V_T 23.1 ± 3.1 L and CL_R 3.6 ± 1.8 mL/min. The within-subject variation of the six parameters in the model varied from 3% for V_{max} to 28% for CL_R. The between-subject variability dominated over the within-subject variation for all parameters except CL_R. Breath samples were associated with similar or lower variation than blood, both within- and between-subjects. V_C was 21% lower in breath compared with blood and 15% lower on day 3 compared with day 1. The within-subject variability was significantly lower in breath for the distribution parameters Cl_d, V_C and V_T.

B-Hb was decreased because of multiple blood sampling and infusions of ethanol-glucose and saline solutions, and there was a reciprocal decrease in fB_{water} (Figure 5). There was a close relationship between *B-Hb* and fB_{water} with an r of 0.94 (Figure 6).

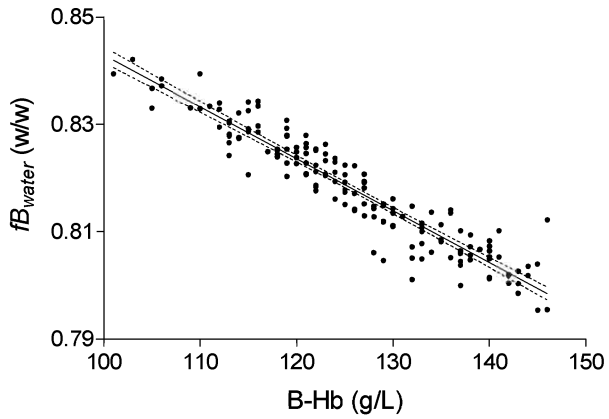

Figure 6
Scatterplot of blood haemoglobin (B-Hb) and the water fraction in whole blood fB_{water} (w/w) for 157 samples from 32 ethanol infusion experiments in 16 healthy subjects (paper IV). The regression equation obtained was $fB_{water} = 0.940 - 0.00097 \cdot$ B-Hb, and Pearson's correlation coefficient $r = 0.94$.

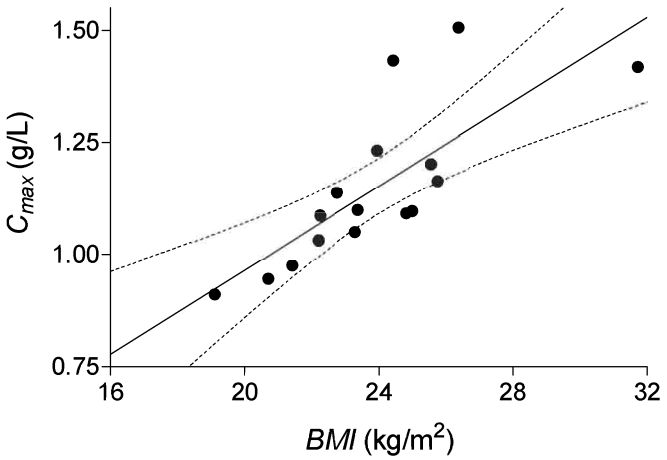

Figure 7
Association between ethanol concentration in expired breath at the end of infusion (C_{max}) and body mass index (BMI) for the 16 subjects of paper V after an ethanol infusion of 0.4 g/kg bw. C_{max} = 0.027 + 0.047 • BMI, Pearson correlation coefficient r = 0.77 and the regression line was significantly different from zero ($p<0.001$).

Paper V

Do ethanol and deuterium oxide distribute into the same water space in healthy volunteers?

Mean *TBW*, determined by D_2O dilution, corresponded to 0.61 L/kg in men and 0.54 L/kg in women. The within-subject coefficient of variation was 2.2-2.6% for ethanol V_{ss} and 1.8% for *TBW* by isotope dilution, corresponding to a 95% confidence interval of ± 1.6 to ± 2.2 L for the applied methods. For the two-compartment model there was a bias of –12.4% (95 CI of mean 10.2-14.6) and –15.6% (13.2-17.9) between V_{ss} determined by ethanol dilution (venous blood and breath, respectively) and *TBW* by D_2O dilution. One-compartment analysis overestimated ethanol V_{ss} by 9% and 13% for venous blood and breath, respectively, compared with the applied two-compartment model. Blood ethanol concentration at the end of infusion (C_{max}) was associated with body mass index (*BMI*) of the subjects (Figure 7), which suggests that the highest ethanol concentrations could be avoided by ethanol dosing by *BMI* instead of by body weight.

DISCUSSION

Pharmacokinetic models for ethanol

Choice of best model

During the course of the work in this thesis, a considerable development in our understanding of the best pharmacokinetic model for ethanol disposition has taken place. We were initially influenced by the generally accepted concept that ethanol was distributed into the total body water and that once this distribution was completed a single compartment model would suffice to describe the terminal phase of the ethanol concentration-time plot.[104, 159] We wanted to improve on earlier results and started to look for factors that might increase the precision of estimating C_0, the y-intercept. Mathematically the error increases with the distance of extrapolation from a linear regression. To get earlier time points one can either decrease the infusion time (paper II) or try to use earlier time points by determining the half-life of the initial (assumed distribution) phase (paper II). It became clear that a 15 min infusion time gave unacceptable side effects such as nausea and inebriation in some subjects with an ethanol dose 0.4 g/kg.

We also tried to improve our estimates by using data from the whole concentration-time profile (papers II and III). These efforts led to a hybrid model (Figure 8). A high coefficient of correlation was obtained between model prediction and concentration-time data. However, with increased pharmacokinetic experience we came to realise that this was not a modern way to proceed because a distribution is always followed by a redistribution and a two-compartment model will never reach a steady state after a short infusion time except for a momentary pseudo-equilibrium when the curves cross some time after the cessation of the infusion.

Finally we arrived at a two-compartment model with parallel Michaelis-Menten and first-order renal elimination, which has a sound theoretical foundation (paper IV and V).

Aspects of one- vs. multi-compartment models

When considering different models to describe the disposition of exogenous compounds in the human body, awareness of some basic physiological processes is of great help. Distribution of any compound can be rate-limited by either permeability or perfusion. If tissue membranes do not constitute a barrier to distribution, perfusion-rate limitation is likely to prevail. The muscles represent a water reservoir for the body comprising about 50% of *TBW*, but the resting muscles only receive 15% of cardiac output at rest, and therefore represent the lowest perfusion per mL tissue water content (Table 7).

Ethanol is a small extremely hydrophilic compound and is believed to use the same transmembrane channels as water,[104] and is therefore likely to follow perfusion-limited kinetics. To consider the whole body as a single well-mixed compartment, as in the one-compartment model, is therefore theoretically doubtful. The initial phase of the concentration-time plot observed in our studies (all papers), and by other investigators after intravenous infusions of ethanol[167, 169, 191] suggests the need for a multi-compartment model for ethanol. In spite of the abundant evidence of multi-compartment kinetics for ethanol only a few studies have applied such kinetic models after intravenous administration, probably because it is difficult to obtain sufficient data for such modelling owing to the very complicated absorption kinetics of ethanol in drinking experiments.[169, 191, 227]

Table 7
The distribution of body water and blood flow in different tissues of a normal 69.4 kg male with a total body water of 40.8 L or 58.5% (w/w) estimated by desiccation.[19]

Tissue [1]	Weight [2]		Tissue water content [2]			Blood flow [3]		
	(kg)	*% (w/w)*	*(kg)*	*% (w/w)*	*% of TBW*	*mL/min*	*% of CO*	*mL/min/mL tissue water*
Blood	3.42	5.0	2.84	83.0	7.0	(5000)	100	-
Bone	11.1	15.9	2.44	22.0	6.0	250	5	0.10
Brain	1.37	2.0	1.03	75.0	2.5	700	14	0.68
Fat	12.6	18.2	3.76	29.9	9.2	200	4	0.053
Kidneys	0.259	0.4	0.214	82.7	0.5	1100	22	5.14
Liver	1.58	2.3	1.08	69.3	2.6	1350	27	1.25
Lungs	0.475	0.7	0.375	79.0	0.9	(5000)	100	13.33
Muscle (resting)	29.1	41.8	22.0	75.7	53.9	750	15	0.034
Skin	4.85	6.9	3.49	72.0	8.6	300	6	0.095

[1] Heart, gut, spleen and miscellaneous organs amount to 6.8% of body weight, 8.8% of total body water and 7% of cardiac output.
[2] Data taken from Bischoff.[19]
[3] CO = cardiac output. Data taken from Rowland and Towser.[234]

Another question is whether C_0 in the one-compartment model is proportional to the ethanol dose. Simulations with different ethanol doses and the achieved mean model parameters of the two-compartment model, using time points from 100 to 480 min and excluding all values below 0.12 g/L to assess C_0 by linear regression, showed that C_0 increases more than expected for the dose given. Thus, the obtained V_{ss} is dose-dependent and overestimated by the lower doses. This is in agreement with the findings of Wagner *et al.*[235] who compared zero-order and Michaelis-Menten elimination from a one-compartment model. In summary the one-compartment model with zero-order elimination and the estimation of V_d from C_0 are based on erroneous assumptions.

The liver as a separate compartment and consequences for parameter estimates

We applied the data from paper IV to a semi-physiologic three-compartment model with elimination from the liver compartment and constant values for the liver water volume 1.1 L and liver blood water flow 1.1 L/min (Figure 8). This seemed physiologically justified and helped to determine model parameters for the liver. Equations 8, 10 and 11 (page 36) were used and the turnover of the central compartment (Equation 9) was modified;

$$V_C \cdot \frac{dC}{dt} = \frac{D_{iv}}{T_{\inf}} - Q_H \cdot C + Q_H \cdot C_H - Cl_d \cdot C + Cl_d \cdot C_T - CL_R \cdot C \qquad (12)$$

The turnover in the liver compartment was;

$$V_H \cdot \frac{dC_H}{dt} = Q_H \cdot C - Q_H \cdot C_H - CL \cdot C_H \qquad (13)$$

where C_H is the concentration in the liver compartment, V_H is the liver water volume and Q_H the liver blood water flow rate.

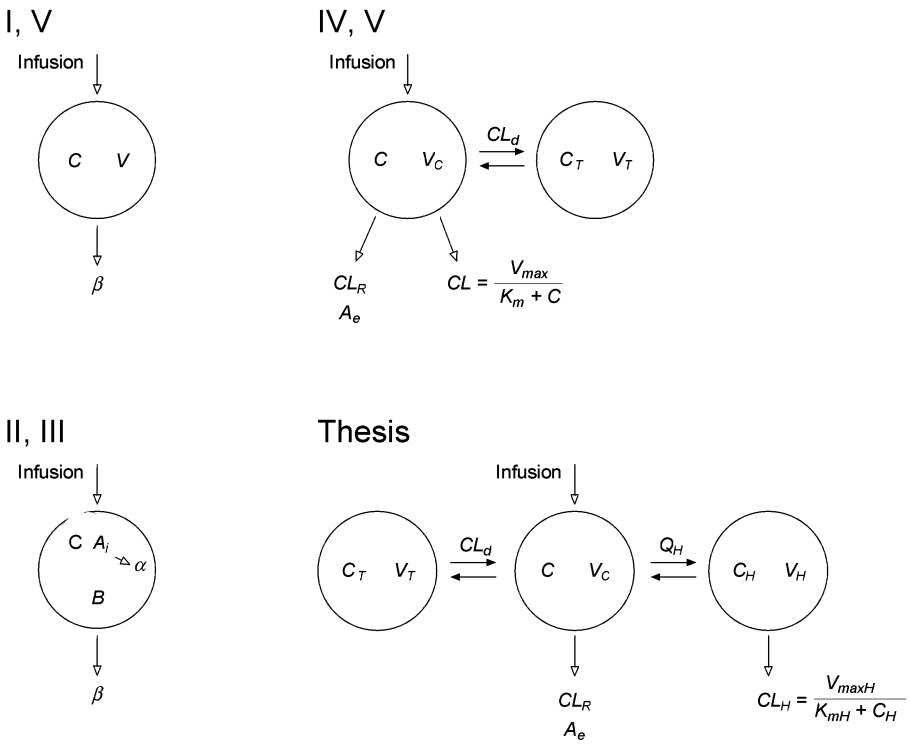

Figure 8
Models of ethanol pharmacokinetics. Roman numbers refer to papers included in this thesis. The three-compartment semi-physiologic model is described on page 44. The parameters V_{maxH} and K_{mH} indicate that they are derived from a model with a separate liver compartment.

Some of the parameters of ethanol kinetics are sensitive to the model used. This is particularly true for K_m (Table 8). Our finding that K_m was reduced to 10% of the systemic value, when the three-compartment model of Figure 8 was applied to the data set of paper IV, agreed with another study where the liver was modelled as a separate compartment.[147] V_{ss} on the other hand was hardly affected at all. Some of the difficulties with ethanol pharmacokinetics can be illustrated by the fact that several investigations report K_m estimates that appear to be erroneous.[144, 196, 227] Furthermore, errors in the measurement units occur repeatedly,[139, 154, 155, 196] and a very strange model with first-order absorption and an elimination that was first-order for 155 min before changing into a Michaelis-Menten elimination for the rest of the time recently appeared in a well-respected journal.[236]

Further aspects of the two-compartment model

To obtain a better understanding of the components of the two-compartment model, the effect of each parameter was studied. Mean estimates of the model parameters from paper IV were used in a simulation of concentration-time profiles where in turn each of the model parameters was altered from mean ± 2SD (Figure 9).

Table 8
V_{max} & K_m values summarized from the literature and compared with paper IV

Reference	*Study design*	*1C, integrated* V_m[2]/K_m	*1C* V_{max}/K_m	*2C* V_{max}/K_m	*liver-C* V_{maxH}/K_{mH}
Wilkinson 1976[228]			78/39		
Vestal 1977[191]	0.57, iv, 50M			95/60	
Rangno 1981[169]	0.4-1.2, iv/po, 8M			156/30	
Minors 1985[195]	0.4-0.8, po,5M6F		/92		
Wagner 1989[237]		183/70	140/70		
Kohlenberg-Muller 1990[238]	0.17, iv, 10MF		108/35		
Pieters 1990[144]	28.5g, 6M6F	475/392			
Levitt 1994[147]					60/2.1
Sharma 1995[196]	0.3, iv, 12M	192/451			
Amir 1996[139]	0.3, iv, 8M	179/95			
Ammon 1996[227]	0.6, iv/po, 6M6F	4782/814			
Gentry 1999[154]	0.3, iv, 6M	191/98			
Mumenthaler 2000[236]	0.69, po, 27F	136/96			
Baraona 2001[155]	0.3, iv, 32M33F	175/118			
Norberg, Paper I	0.4, iv, 6M6F fasted fed			105/17 133/17	
Norberg, Paper IV	0.4, iv, 8M8F		104/35	94/24	89/2.9

[1] Ethanol dose per kg *bw*, route of administration, number of subjects and gender.
[2] V_m, has the unit mg/L/h as opposed to V_{max} that has mg/min.
[3] Liver-C means that the values are derived from a model where the liver constitutes a separate compartment, and the rest of the body is either one (Levitt) or two (Norberg, thesis page 43) compartments.

Whereas CL_R lacks importance it is noteworthy that changes in V_{max} and K_m can produce similar effects on the concentration-time profile. The assumptions of the two-compartment model were scrutinized:

Two well-stirred compartments are sufficient to describe ethanol pharmacokinetics and calculate V_{ss} with a high precision. The major problem with introducing more compartments is the increased number of model parameters. This results in a decreased precision of the parameter estimates. The data sets of paper IV were re-analysed with a model containing a third peripheral compartment but precise parameter estimates could not be obtained.

Cl_d is constant during the measurement period. This is a weak point of the model. Any muscular activity is likely to change Cl_d, as will stress-induced vasoconstriction or a rise in body temperature. Ethanol itself might influence cardiac output or local circulation in muscle and thus indirectly Cl_d.

CL_R is constant and the first-order elimination is an appropriate model of renal elimination. Ethanol consistently increases renal water excretion.[239-241] Each gram of ethanol induces diuresis of about 10 mL, but the variability is great and related to the rapid rise in blood alcohol concentration.[239] The strong diuresis persists for 1-3 hours after end of drinking and is followed by anti-diuresis.[242] Plasma osmolality contributes to the ethanol diuresis.[243]

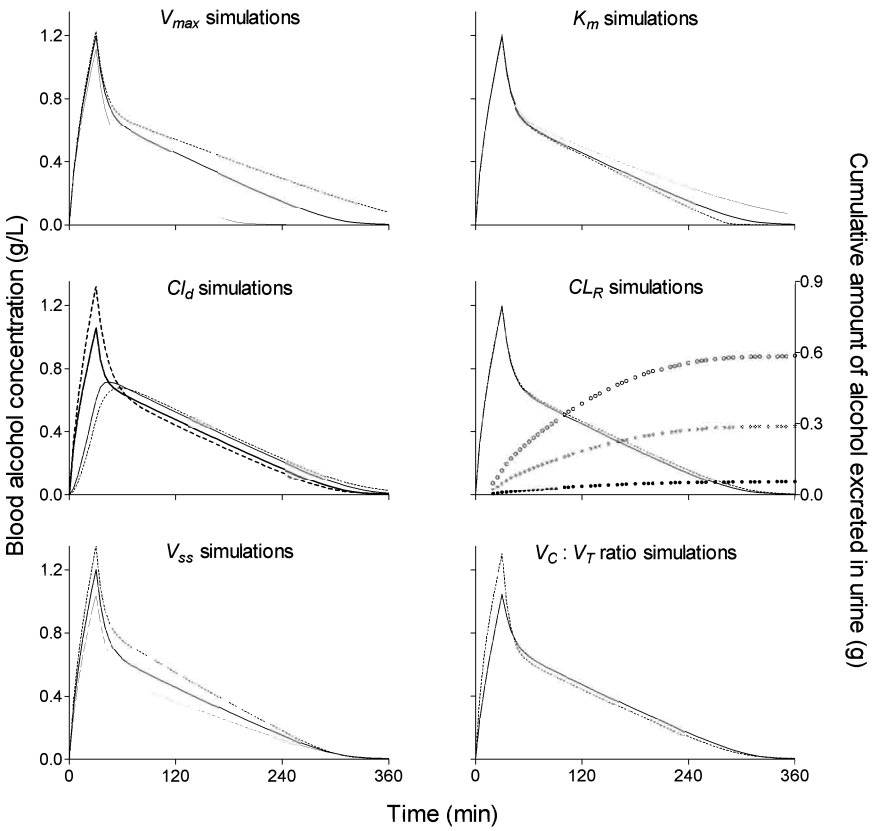

Figure 9
Simulated impact on the concentration-time plot by each parameter in the two-compartment model with parallel Michaelis-Menten and first-order renal elimination. Mean parameter – 2 SD (dotted lines) and + 2SD (hair lines)

Ethanol's diuretic effect is thought to involve inhibition of the antidiuretic hormone plasma arginin vasopressin,[243, 244] but others have failed to confirm this finding.[242, 245] Basal fluid balance seems to be crucial for the hormonal response to ethanol. Well-hydrated subjects had increased urine output after an ethanol dose of 1.0 g/kg. However, no changes were observed in plasma atrial natriuretic factor, plasma arginin vasopressin or plasma renin activity with either ethanol or orange juice.[245] Noradrenergic mechanisms may be involved in ethanol induced diuresis.[246] In summary less than 5% of the ethanol dose is eliminated by renal excretion. Because of this limited contribution to total elimination, an increase in the number of parameters for renal elimination is not motivated. Furthermore, scrutinizing of data suggests that the diuretic response to ethanol does not follow first-order elimination in all cases (paper IV).

The maximal metabolic rate (V_{max}) is constant during the measurement period. This issue will be covered in detail below, although it is likely that the changes in metabolism induced by feeding has a gradual onset that is not considered by the present model.

Experimental factors influencing model parameters

Feeding state

Eating a meal accelerates ethanol metabolism by about 30% even when the intravenous route is used (paper I). A recalculation of data with the more appropriate two-compartment model and Michaelis-Menten elimination kinetics confirmed this finding. This effect is difficult to detect in drinking experiments. Two recent studies[173, 247] made use of breath ethanol clamping of intravenous ethanol infusions to investigate the effect of food on ethanol metabolic rate. They reported increases in *AER* in accordance with paper I of this thesis. This "food-effect" phenomenon has also been reported for other drugs, and food-induced alteration in liver blood flow has been suggested as a possible mechanism.[248]

Liver blood flow, Q_H

If more than 70% of a compound is extracted from the blood during a single pass through the liver it is called a high extraction compound (E_H > 70%), and the liver blood flow rate will be an important determinant of clearance of the drug. Ethanol is such a high clearance compound at low concentrations and it has even been suggested as a tool to estimate liver blood flow (Q_H) from infusions of low doses of ethanol.[249, 250] Hepatic elimination can be estimated from the following equation:

$$CL_H = \frac{Q_H \cdot CL_{\text{int}}}{Q_H + CL_{\text{int}}} \qquad (14)$$

If liver blood flow (Q_H) is increased there will be an increase in the elimination of ethanol provided C_H is low. However, hepatic clearance of ethanol is also concentration dependent. Intrinsic clearance (CL_{int}) can be defined as the flow-independent maximal enzymatic rate of elimination, and can be calculated according to the following equation:

$$CL_{\text{int}} = \frac{V_{\max H}}{K_{mH} + C_H} \qquad (15)$$

V_{maxH} and K_{mH} are parameters derived from a model with the liver as a separate compartment. In the two-compartment model the apparent CL_{int} was about 4 L/min and in the 3-compartment semi-physiological model it was 30 L/min, assuming a constant Q_H of 1.1 L/min and C_H = 0. With these figures a 50% increase in Q_H to 1.65 L increases CL_H by 16% and 6% at alcohol concentrations of 0.1 and 0.4 g/L, respectively. Such an increase in Q_H in association with eating a meal is reasonable,[248] but cannot alone explain the difference in elimination rate between the fed and fasted states in all cases. The combined effects of the ethanol concentration in the liver and liver blood flow on hepatic elimination rate of ethanol are shown in Figure 10. Another complicating factor is that ethanol itself seems to increase liver blood flow.[251]

Intrinsic clearance

Another mechanism that might accelerate metabolism of ethanol in the fed state is an increase of V_{max} that can be achieved either by an increased amount of enzymes or by a change in co-factors such as the rate of re-oxidation of NADH or decreased activity of ALDH. Rats deprived of food for 24 hours exhibited a reduction of the liver size with a concurrent 40% decrease in

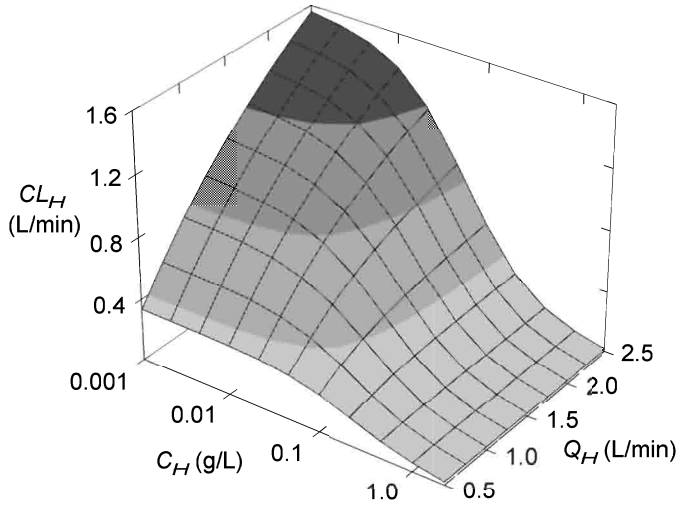

Figure 10
Hepatic clearance (CL_H) as a function of hepatic blood alcohol concentration (C_H) and hepatic blood water flow (Q_H). The mean values from paper IV were used in the calculations (V_{max} 0.095 g/min and K_m 0.0245 g/L).

ADH activity.[252] The higher basal metabolic rate and shorter life cycle of rodents makes the scaling of such results to humans difficult.

We observed a significant difference in metabolic rate after only 5-10 hrs of fasting (paper I). However, it cannot be ruled out that fasting lowers the amount of active enzyme for ADH, ALDH or some co-factor and that they amount to a significant part of the decreased ethanol metabolic rate in the fasted state in humans.

Temperature and vasoconstriction

We found that heating of the hand decreased but did not abolish the arterio-venous difference in blood ethanol concentrations (paper III). Such ethanol experiments have not been performed earlier, and the degrees of successful arterialization for other compounds are variable.[220, 253]

We had observed that the distribution phase could be almost abolished in certain situations and we interpreted this as a result of stress induced vasoconstriction.[254] Local cooling increased the arterio-venous difference in ethanol concentrations in healthy male volunteers but a prominent distribution phase was preserved in all subjects (paper III). The difference between the apparent volume of distribution from arterial and venous blood increased in the cooling experiments. Peripheral vasoconstriction was thus identified as a confounding factor for correct estimation of ethanol V_{ss}. The mechanism was not clarified, but we believe that a decreased blood flow rate alone can cause this effect. A high degree of arterio-venous shunting would have resulted in a decreased arterio-venous difference.

In conclusion, decreased peripheral circulation impairs the representativness of venous blood for the whole blood volume and should warn the investigator that estimates of pharmacokinetic parameters obtained might be erroneous.

Miscellaneous

Oral versus intravenous administration of ethanol

We thought that intravenous administration of ethanol would improve the precision and repeatability of the V_{ss} estimation. We performed data-simulations to investigate the effects of gastric emptying rate and lag-time on the *VBAC*-time profile. No FPM was anticipated, but nevertheless a slow emptying decreases the available time span of the pseudolinear part of the *VBAC*-time plot and a lag-time of 5 minutes increases C_0 by 3%. With low doses of ethanol the FPM seems to be an important factor influencing inter- and intraindividual variability.[147]

Some efforts to model ethanol absorption kinetics exist, but stable parameter estimates could not be obtained.[131, 144] The highly variable gastric emptying rate, absorption from the stomach and first-pass metabolism in the liver and/or gastric mucosa requires high-resolution data of several sources.

The ethanol dose, infusion rate and inebriation

Several investigators report dropouts in ethanol experiments because of inebriation and nausea.[191, 228, 255] An ethanol dose of 0.4 g/kg *bw* consumed rapidly by elderly women made some of the subjects unable to provide breath ethanol measurements.[255] Occasionally a higher dose of 0.8 g/kg *bw* orally has been reported to cause no adverse side-effects, but perhaps these subjects were accustomed to a higher ethanol consumption.[203]

The dose of 0.4 g/kg in 15 minutes caused unacceptable side-effects in some subjects as determined by the visual analogue scale (paper II). The same dose as a 30 min infusion therefore became our choice to obtain a prominent distribution phase, a reasonably long sampling time period and a minimum of side effects. An adjustment of the ethanol dose according to an anthropometric estimation of *TBW* would avoid the highest transient blood ethanol levels in subjects with a high body mass index (Figure 7).

Subjective feeling of inebriation by a visual analogue scale (VAS) proved to be a very blunt instrument for assessment of *VBAC*. VAS 30 min after the end of the ethanol infusion ranged from 2.5 to 9.5 when *VBAC* varied from 0.32 to 0.57 g/L (paper II). Similar large variability has also been reported using more sophisticated sedation indexes after ethanol administration.[227]

Subject selection

The choice of subjects is of great importance in all ethanol pharmacokinetic experiments. The impact of gender on peak ethanol concentrations and elimination rates has been much investigated.[155, 188, 189, 199] Women exhibit a lower value for V_{ss} compared with men, something that was confirmed in paper IV and found to relate to a lower *TBW* (paper V). We did not find a gender related difference in V_{max}, but others have found significant differences in k_0 or *AER*.[188, 189] Ethanol elimination rate decreases with age,[191] and the volume of distribution relates to age dependent changes in *TBW*.[190] Our subjects did not cover the age-span where age dependency is likely to become significant.

We did all our experiments in healthy volunteers, although our two-compartment model for ethanol pharmacokinetics should be valid for other subjects. However, the size of the bias between ethanol V_{ss} and *TBW* by isotope dilution might vary with disease, ethnicity, or old age, and these factors may prove to be important for estimation of V_{ss} and even for the optimal experimental design.

Site of measurement

The vascular system

The interpretation of arterial and venous blood sampling in pharmacokinetics has been reviewed by Chiou.[256, 257] He stressed that arterial blood is a reflection of the whole body pharmacokinetics, whereas venous blood represents the sum of the whole body process and the local absorption, distribution and elimination in the tissues drained by the particular sampling vein. In the case of ethanol the distribution and re-distribution will show local variations because of different blood flow rates and tissue water content. Factors that govern the local blood flow or tissue hydration is therefore likely to alter the arterio-venous difference.

We found that the arterio-venous difference changed direction within 5 minutes of ending the ethanol infusion (paper III). This finding suggests that ethanol pharmacokinetics is governed by perfusion rather than by permeability. Others have also reported changes in ethanol concentrations between different segments of the vascular system.[143, 167, 258, 259] During absorption the following relations of blood alcohol concentrations were obtained: arterial > capillary > cubital vein > hand vein. In the post-absorptive phase the relationships were reversed: arterial < capillary < cubital vein. Our results (paper III) confirm this notion, and suggest that a single well-mixed compartment is insufficient to explain ethanol disposition. As noted in the cold hand experiment (paper III) pharmacokinetic parameters can be affected by the choice of sampling site in the vascular system. In paper IV two venous blood concentration-time profiles did not provide sufficient data for the two-compartment analysis, again stressing the vulnerability of venous blood sampling to factors such as stress-induced vasoconstriction.

Variations in blood water content can be handled by transforming the concentrations of ethanol in whole blood into concentrations in the water fraction of the blood (paper IV and V). However, this is hardly necessary unless a very accurate estimate of V_{ss} is required. Instead of measuring blood water content by desiccation, the close relationship between blood haemoglobin and blood water can be used (Figure 5). The problem of variable haematocrit can be handled by analysis of plasma ethanol, because the variability of the water fraction of plasma is much less. Pharmacokinteic parameters will be affected by the choice of body fluid (Table 9). Other investigators have used the haematocrit instead of haemoglobin to form relations with the blood water content.[260, 261] Values in the literature were used for the density and water content of red blood cells and plasma.[223, 224] However, one still needs to assume that there were no appreciable changes in red cell haemoglobin concentration. The validity of this assumption can be questioned especially under pathological conditions such as uraemia, congestive heart failure or in the ICU because a rise in blood water is likely to cause swelling of the erythrocyte with a concomitant fall in red cell haemoglobin concentration.

Density is defined as mass/volume and this is a temperature dependent unit as most compounds increase in volume if the temperature is raised and thus density decreases. If the specimen is measured at a temperature of 20°C and referred to the density of water at 4°C this is noted d^4_{20}. This means that the temperature must be considered if a correct transformation of whole blood alcohol concentration to alcohol in the water fraction of the blood is required. The magnitude of error that can be introduced is about 2%.[225]

Table 9

Pharmacokinetic parameters are affected by the choice of specimen analysed and by the particular blood breath ratio (BBR) used. The correction factors are obtained by simulation in classical analysis, and in a two-compartment model with parallel Michaelis-Menten and renal first-order elimination. Whole blood and breath-alcohol agree well when a BBR of 2300 is used. Blood density was assumed to be 1.055 g/L at a blood water fraction of 80% (w/w), and plasma density 1.027 g/L at a plasma water content of 92% (w/w).[262]

Pharmacokinetic parameter	Whole blood *BBR* 2300 *(g/L)*	Plasma *(g/L)*	Blood water *(g/L)*	*BBR* 2100 *(g/L)*
C, C_{max} and C_0 (g/L) [1]	1.0	1.11	1.19	0.91
k_0 (g/L/h) [1]	1.0	1.11	1.19	0.91
AUC (g•h/L) [1]	1.0	1.11	1.19	0.91
V_d (L)	1.0	0.90	0.84	1.10
V_{max} (g/h) [1]	1.0	1.0	1.0	1.0
K_m (g/L) [1]	1.0	1.11	1.19	0.91
V_C, V_T and V_{ss} (L)	1.0	0.90	0.84	1.10
Cl_d and CL_R (L/min)	1.0	0.90	0.84	1.10

[1] If mmol is used instead of g ethanol, the parameters will require a correction factor of 21.7.

The blood:breath ratio for ethanol

We report that breath analysis correlated well with venous blood-alcohol for estimating pharmacokinetic parameters of ethanol (paper IV and V). We used a blood-breath ratio of 2300:1 (paper IV and V), and the influence of the chosen ratio on the kinetic parameters was investigated by simulations with different ratios (Table 9). The lower V_C reported for breath data (paper IV) is in accordance with the strong correlation between breath and arterial blood alcohol (paper III) suggesting a centralisation of arterial kinetics. V_{ss} was very similar in venous blood and breath with the chosen blood-breath ratio (paper V). We took great care to standardize the exhalations, which might have contributed to the good results obtained. It is also important to note that excellent breath concentration-time profiles were obtained for the two outliers in paper IV, whereas the venous blood sampling failed to provide sufficient data for the two-compartment analysis.

According to pharmacokinetic theory the total amount of a compound entering a certain tissue and the total amount leaving it must be identical if there is no significant metabolism or elimination in the region drained by the sampling vein. If breath ethanol is considered an index of arterial blood ethanol it should be possible to compare AUCs from breath and venous blood sampling to determine the blood:breath ratio of a particular subject. We found a mean value of 2200 in the subjects participating in paper IV corresponding to a CV of 5.1%. However, the variability of the estimated V_{ss} in paper V was not improved by this procedure.

Local blood flow related to Cl_d might not be constant during the sampling time, but more importantly the venous blood:breath ratio is not constant during an intravenous ethanol experiment (Figure 11). There is a continuous increase of the venous blood:breath ethanol ratio, and further model development is necessary to explain and interpret these differences.

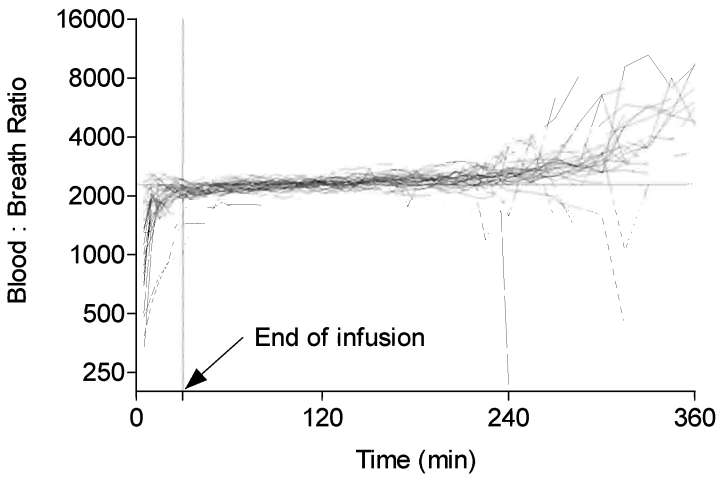

Figure 11
Spaghetti plot showing the variation of venous blood:breath ratio of ethanol following a 30-min infusion of 0.4 g/kg body weight. Data from 32 experiments (paper IV).

Total body water by ethanol dilution

Precision

The precision of estimating V_{ss} for ethanol almost reached 2% for the two-compartment model with parallel Michaelis-Menten and first-order renal elimination (paper IV and V), which is similar to the precision of the isotope dilution method for estimating *TBW* (paper V). Breath measurements were at least as good as venous blood in providing repeatable results.

Based on the results of paper II, we opted for an ethanol dose of 0.4 g/kg *bw* given as an intravenous infusion in 30 min, to avoid the side-effect of inebriation and still get enough data points to obtain precise estimates of the multi-compartment parameters of ethanol kinetics. With the intravenous route all problems associated with the complicated absorption kinetics of ethanol were avoided.

In previous investigations of the ethanol dilution technique a single experiment was performed in each subject (Table 4), with the exception of Dahl *et al.*[204] who determined the ethanol space a second time in 17 subjects after 2-30 days. Bland-Altman analysis showed a mean difference of -4 mL/kg with 95% limits of agreement of -40 and 32 mL/kg corresponding to a SD of the difference of about 17 mL/kg or about 3% of the estimated *TBW*. This precision is slightly less than the 2.2% obtained using classical analysis based on zero-order elimination kinetics (paper V). The statistical analysis of precision is covered in some detail in the statistics section (page 37).

Accuracy of two-compartment analysis

To our disappointment, the high precision of the ethanol V_{ss} with the two-compartment model was accompanied by a bias of –12.5% or –15.7% for venous blood and breath measurements, respectively, compared with the *TBW* assessed by isotope dilution. Two other studies have used

a similar two-compartment analysis for ethanol kinetics, but did not primarily address the issue of *TBW*. However, V_{ss} was assessed with high precision, and data were provided in good agreement with our findings. Rangno *et al.*[169] showed that V_d was overestimated by 17% by the one-compartment analysis, where we found a 9% overestimation. However, their subjects were permitted to eat during the sampling time and the figure of 17% was an average of oral and intravenous studies. Vestal *et al.*[191] reported both *LBM* and ethanol V_{ss}. By assuming $LBM \approx FFM$ the relationship $TBW = 0.732 \cdot FFM$ could be used. The ratio between ethanol V_{ss} and this calculated *TBW*-value was 0.815 ± 0.093 which compares well with our values of 0.844 and 0.876 for breath and venous blood, respectively.

Previous investigations of the relationship between V_{ss} and TBW

All previous studies of ethanol dilution as an index of *TBW* relied on the classical one-compartment analysis with zero-order elimination, and the dose of ethanol was administered orally (Table 4).[65, 200, 201, 203-208] The values presented did not differ from the expected *TBW*, and the studies that compared ethanol V_d with *TBW* assessed by antipyrine[207], D_2O[203] or anthropometry[208] all show good agreement between the ethanol V_d and the reference method. The bias reported between the one-compartment analysis in paper V and *TBW* is slightly greater than previous reports. This difference might be caused by use of the intravenous route, the 6% difference when blood water is estimated as vol/vol instead of w/w, differences in ethanol dose, and the rigorous control of other confounding factors in our study. However, we feel that the two-compartment model is superior to the one-compartment model for predicting ethanol kinetics after intravenous infusion in spite of the greater bias between ethanol V_{ss} and *TBW* by isotope dilution.

The ratio between tissue ethanol and whole blood ethanol

To explore if ethanol is a suitable marker for the total body water as compared to deuterium oxide dilution the relationship in Figure 10 can be considered. The relationship between deuterium oxide space and *TBW* has been well investigated by others (Figure 12, III and IV, see pages 14-15). During much of this thesis work we focused on different pharmacokinetic models and their implications for the determination of V_{ss} (Figure 12, I). The bias between V_{ss} by the two-compartment model with parallel Michaelis-Menten and first-order renal elimination and *TBW* by D_2O dilution turned our interest to the basis for the generally

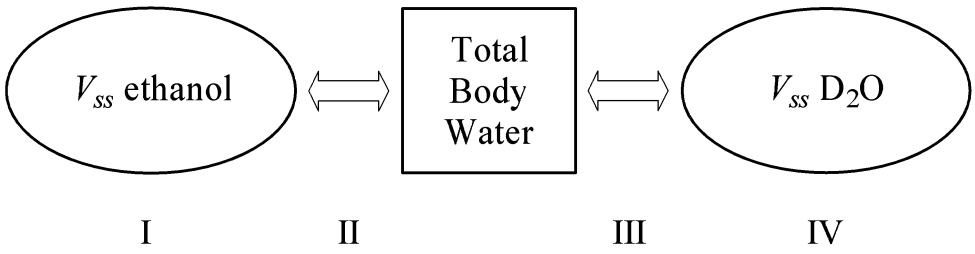

Figure 12
Relationships between total body water and the distribution volumes for ethanol and deuterium oxide, respectively, that need to be considered for an ethanol dilution method and validation experiments.

accepted assumption that "ethanol is distributed into the total body water" (Figure 12, II) that is one of the three main features of ethanol kinetics.[159] The affinity of ethanol to other substances in blood is low.[212] Furthermore, the ratio between whole blood and plasma ethanol concentrations are similar to the ratio between whole blood water and plasma water.[210-212] However, blood does not necessarily represent the whole body water pool. It is the skeletal muscles that contain most of the *TBW* and therefore the comparison between water content and ethanol content should be performed in that tissue. The partition coefficient for ethanol between blood and skeletal muscle has been reported in vitro as 0.80 in rats[263, 264] and 0.64 in humans[265] whereas the muscle/blood ratio of water in humans is about 0.9. The only experiment that reports muscle ethanol *in vivo* did not report the relationship to blood ethanol concentrations but according to two illustrations a ratio of 0.65 can be deduced.[266]

The discussion of brain tissue:blood ethanol concentrations in literature elucidates some of the difficulties with *in vivo* studies, such as measuring in the dynamic state of non-equilibrium, great regional variability in ethanol concentrations between different brain regions, and the use of small rodents that only permit one sampling time point per individual.[267-269]

Even if the exact mechanism and nature of the difference between the ethanol space and *TBW* is not solved by these experiments, our results, as well as the results of others, suggests that ethanol is not distributed into the *TBW*.

Properties of biological water

Biological water has been reported to be structured to a great extent,[270] and extracellular water has been described as a gel rather than as a free fluid.[271] Up to 8 layers of water can cover a biological surface by electrostatic bindings.[272] Water can also form hydrogen bounds on two positions and form hydrostatically unresponsive pentagons over hydrophobic protein domains.[273] Cell activity has been suggested to depend on changes in the relationship between high- and low-density water that probably correlate to the issue of structured water.[274]

In contrast to water, ethanol has only one position for a hydrogen bond and a capability to bind simultaneously to hydrophilic and hydrophobic membrane targets. Different solvent properties of ethanol and water have been reported.[275] The mechanisms of ethanol action include partitioning into the lipid bilayers of membranes,[276] allosteric changes of protein conformation at receptor sites,[277] or dehydration of the cell-surface microdomain owing to hydrophilic properties.[278] In summary, it seems likely that water and ethanol molecules might not be freely exchangeable in all biological tissues, owing to their different polar, steric and solvent attributes.

Considerations for the ICU setting

Our vision to determine *TBW* by ethanol dilution in the ICU setting carries a number of difficulties that were not investigated in the work for this thesis. During the acute phase of critical illness circulation is often supported by vasoactive drugs. Bleeding or capillary leakage can cause loss of tracer and considerable variations in liver blood flow. Huge amounts of intravenous fluids and blood components might be infused. Circulatory stability is often developed after some time and the foremost benefit of the method should be in patients with a longer duration of critical illness with wasting of muscle tissue. Only a few studies on body composition in critical illness have been published,[12, 13, 49, 100] probably because of the methodological and ethical difficulties.

Furthermore, the physiological side effects of administering ethanol also need to be carefully considered. Ethanol is probably the most self-prescribed drug, and a considerable number of the ICU patients are critically ill because of injuries caused either directly or indirectly by over-consumption of ethanol. Physiological effects of chronic alcoholism have been repeatedly reviewed.[279, 280] After the small amounts of ethanol used in this thesis acute effects are very limited in healthy subjects, but they might be important in certain states of critical illness such as liver disease and in severe cardiac arrhythmias.[281, 282]

Pharmacokinetic parameters from breath sampling could be determined with a high precision. Therefore, the bias between the ethanol dilution method and *TBW* by isotope dilution might be investigated in critically ill subjects on ventilator treatment by some device for automated breath sampling. However, many issues need to be addressed and the practical usefulness remains to be determined.

CONCLUSIONS

The work described in this thesis comprises clinical studies with healthy volunteers who were given ethanol by intravenous infusions in doses of 0.4-0.6 g/kg body weight in 15-60 minutes. The results obtained allow the following conclusions regarding the distribution and elimination kinetics of ethanol after intravenous infusion:

- A two-compartment model with parallel Michaelis-Menten and first-order renal elimination gives an excellent fit to ethanol concentration-time data in breath, venous blood and urine after intravenous infusion.

- The classical one-compartment zero-order elimination model is based on erroneous assumptions, and overestimates the ethanol V_{ss} obtained by two-compartment analysis by about 9% when blood specimens were analysed.

- The rate of elimination of ethanol from blood was increased by about 30% after subjects had eaten a meal, even though the ethanol was administered by intravenous infusion.

- Heating the hand used to draw blood samples reduced, but did not abolish, the arterio-venous differences in ethanol concentration.

- Local vasoconstriction induced by immersion of the hand used to sample blood in cold water confounds the pharmacokinetic parameters of ethanol and exaggerates the arterial-venous differences in concentration.

- Breath ethanol concentrations were highly correlated with arterial blood ethanol concentrations. Breath measurements can be as good as blood measurements for pharmacokinetic analysis and can provide an estimate of ethanol V_{ss} at the bedside.

- The rate of intravenous administration of ethanol had a profound effect on alcohol-induced inebriation with more rapid rates producing greater effects on the individual. Giving a dose of 0.4 g/kg *bw* in 15 min produces undesirable side effects in some subjects, but giving the same dose in 30-45 minutes was well tolerated.

- Ethanol V_{ss} can be determined with high precision (CV 2%) after a single intravenous infusion.

- There is a bias of approximately -13% between V_{ss} for ethanol and *TBW* as determined by D_2O when a correction was made for exchange with non-aqueous hydrogen.

- An ethanol dilution method for determination of *TBW* in critically ill patients could not be established because of an unexpected bias between V_{ss} and *TBW* by water isotope dilution. The nature, variability and physiological relevance of the difference between *TBW* and ethanol V_{ss} remains to be explored.

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REFERENCES

1. Wang ZM, Pierson RN, Jr., Heymsfield SB. The five-level model: a new approach to organizing body-composition research. *Am J Clin Nutr* 1992; 56: 19-28.

2. Pace N, Rathbun EN. Studies on the body composition. III. The body water and chemically combined nitrogen content in realtion to fat content. *J Biol Chem* 1945; 158: 685-691.

3. Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: review and critique of a classic body-composition constant. *Am J Clin Nutr* 1999; 69: 833-841.

4. Roche AF: Anthropometry and ultrasound, in Roche AF, Heymsfield SB, Lohman TG (eds): *Human Body Composition.* Champaign, IL, Human Kinetics, 1996, 167-190.

5. Ellis KJ. Human body composition: in vivo methods. *Physiol Rev* 2000; 80: 649-680.

6. Brodie D, Moscrip V, Hutcheon R. Body composition measurement: a review of hydrodensitometry, anthropometry, and impedance methods. *Nutrition* 1998; 14: 296-310.

7. Heymsfield SB, Wang Z, Baumgartner RN, Ross R. Human body composition: advances in models and methods. *Annu Rev Nutr* 1997; 17: 527-558.

8. Roche AF, Heymsfield S, Lohman TG: *Human body composition.* Champaign, IL, Human Kinetics, 1996.

9. Sutcliffe JF. A review of in vivo experimental methods to determine the composition of the human body. *Phys Med Biol* 1996; 41: 791-833.

10. Sheng HP, Huggins RA. A review of body composition studies with emphasis on total body water and fat. *Am J Clin Nutr* 1979; 32: 630-647.

11. Schober O, Ollech J, Lehr L, Hundeshagen H. Der Quotient Ganzkörperkalium/ Ganzkörperwasser als Maß für die mitlere intrazelluläre Kaliumkonzentration. *Nuklearmedizin* 1981; 20: 220-228.

12. Cheng AT, Plank LD, Hill GL. Prolonged overexpansion of extracellular water in elderly patients with sepsis. *Arch Surg* 1998; 133: 745-751.

13. Finn PJ, Plank LD, Clark MA, Connolly AB, Hill GL. Progressive cellular dehydration and proteolysis in critically ill patients. *Lancet* 1996; 347: 654-656.

14. Schoeller DA: Hydrometry, in Roche AF, Heymsfield SB, Lohman TG (eds): *Human Body Composition.* Champaign, IL, Human Kinetics, 1996, 25-44.

15. Southgate DA. Body content and distribution of water in healthy individuals. *Bibl Nutr Dieta* 1987; 40: 108-116.

16. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of ^{2}H- and ^{18}O-labeled water in humans. *Am J Physiol* 1994; 267: E585-590.

17. Bolton MP, Ward LC, Khan A, Campbell I, Nightingale P, Dewit O, Elia M. Sources of error in bioimpedance spectroscopy. *Physiol Meas* 1998; 19: 235-245.

18. Tagliaro F, Lubli G, Ghielmi S, Franchi D, Marigo M. Chromatographic methods for blood alcohol determination. *J Chromatogr* 1992; 580: 161-190.

19. Bischoff E. Einige Gewichts- und Trocken-Bestimmungen der Organe des menschlichen Körpers. *Zr Rat Med* 1863; 20: 75-118.

20. Mitchell HH, Hamilton TS, Steggerda FR, Bean HW. The chemical composition of the adult human body and its bearing on the biochemistry of growth. *J Biol Chem* 1945; 158: 625-637.

21. Widdowson EM, McCance RA, Maxwell WB, Coldwell BB, Trenholm HL. The chemical composition of the human body. *Clin Sci* 1951; 10: 113-125.

22. Forbes RM, Cooper AR, Mitchell HH. The composition of the adult human body as determined by biochemical analysis. *J Biol Chem* 1953; 203: 359-366.

23. Forbes GB, Lewis AM. Total sodium, potassium and chloride in adult man. *J Clin Invest* 1956; 35: 596-600.

24. Forbes RM, Mitchell HH, Cooper AR. Further studies on the gross composition and mineral elements of the adult human body. *J Biol Chem* 1956; 223: 969-975.

25. Moore FD, Lister J, Boyden CM, Ball MR, Sullivan N, Dagher FJ. The skeleton as a feature of body composition. Values predicted by isotope dilution and observed by cadaver dissection in an adult human female. *Hum Biol* 1968; 40: 135-188.

26. Knight GS, Beddoe AH, Streat SJ, Hill GL. Body composition of two human cadavers by neutron activation and chemical analysis. *Am J Physiol* 1986; 250: E179-185.

27. Lofti M, Macdonald IA, Stock MJ. Energy losses associated with oven-drying and the preparation of rat carcasses for analysis. *Br J Nutr* 1976; 36: 305-309.

28. Lewin S: *Displacement of water and its control of biochemical reactions*. London, Academic Press, 1974.

29. Culebras JM, Fitzpatrick GF, Brennan MF, Boyden CM, Moore FD. Total body water and the exchangeable hydrogen. II. A review of comparative data from animals based on isotope dilution and desiccation, with a report of new data from the rat. *Am J Physiol* 1977; 232: R60-65.

30. Urey HC, Brickwedde FG, Murphy GM. A hydrogen isotope of mass 2. *Phys Rev* 1932; 39: 164-165.

31. Alvarez LW, Cornog R. Helium and hydrogen of mass 3. *Phys Rev* 1939; 56: 613.

32. Hevesy G, Hofer E. Elimination of water from the human body. *Nature* 1934; 134: 879.

33. Moore FD. Determination of total body water and solids with isotopes. *Science* 1946; 104: 157-160.

34. Pace N, Kline L, Schachman HK, Harfenist M. Studies on body composition IV: Use of radioactive hydrogen for measurement in vivo of total body water. *J Biol Chem* 1947; 168: 459-469.

35. Burkholder WJ, Thatcher CD. Validation of predictive equations for use of deuterium oxide dilution to determine body composition of dogs. *Am J Vet Res* 1998; 59: 927-937.

36. Rothwell NJ, Stock MJ. In vivo determination of body composition by tritium dilution in the rat. *Br J Nutr* 1979; 41: 625-628.

37. Whyte RK, Bayley HS, Schwarcz HP. The measurement of whole body water by H2(18)O dilution in newborn pigs. *Am J Clin Nutr* 1985; 41: 801-809.

38. Tisavipat A, Vibulsreth S, Sheng HP, Huggins RA. Total body water measured by desiccation and by tritiated water in adult rats. *J Appl Physiol* 1974; 37: 699-701.

39. Kanto U, Clawson AJ. Use of deuterium oxide for the in vivo prediction of body composition in female rats in various physiological states. *J Nutr* 1980; 110: 1840-1848.

40. Culebras JM, Moore FD. Total body water and the exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. *Am J Physiol* 1977; 232: R54-59.

41. Wong WW, Cochran WJ, Klish WJ, Smith EO, Lee LS, Klein PD. In vivo isotope-fractionation factors and the measurement of deuterium- and oxygen-18-dilution spaces from plasma, urine, saliva, respiratory water vapor, and carbon dioxide. *Am J Clin Nutr* 1988; 47: 1-6.

42. Wentzel AD, Iacono JM, Allen TH, Roberts JE. Determination of heavy water (HDO) in body fluids by direct introduction of water with a mass spectrometer. *Physiol Med Biol* 1958; 3: 1-6.

43. Nielson WC, Krzywicki HJ, Johnson HL, Consolazio CF. Use and evaluation of gas chromatography for determination of deuterium in body fluid. *J Appl Physiol* 1971; 31: 957-961.

44. Lukaski HC, Johnson PE. A simple, inexpensive method of determining total body water using a tracer dose of D2O and infrared absorption of biological fluids. *Am J Clin Nutr* 1985; 41: 363-370.

45. Khaled MA, Lukaski HC, Watkins CL. Determination of total body water by deuterium NMR. *Am J Clin Nutr* 1987; 45: 1-6.

46. Halliday D, Miller AG. Precise measurement of total body water using trace quantities of deuterium oxide. *Biomed Mass Spectrom* 1977; 4: 82-87.

47. Schoeller DA, van Santen E, Peterson DW, Dietz W, Jaspan J, Klein PD. Total body water measurement in humans with 18O and 2H labeled water. *Am J Clin Nutr* 1980; 33: 2686-2693.

48. O'Meara MP, Birkenfeld LW, Gotch FA. The equilibration of radiosodium (Na^{24}), radiopotassium (K^{40}), and deuterium oxide (D_2O) in hydropic human subjects. *J Clin Invest* 1957; 36: 784-792.

49. Streat SJ, Beddoe AH, Hill GL. Measurement of total body water in intensive care patients with fluid overload. *Metabolism* 1985; 34: 688-694.

50. Bratteby LE, Sandhagen B, Fan H, Enghardt H, Samuelson G. Total energy expenditure and physical activity as assessed by the doubly labeled water method in Swedish adolescents in whom energy intake was underestimated by 7-d diet records. *Am J Clin Nutr* 1998; 67: 905-911.

51. Schloerb PR, Friis-Hansen BJ, Edelman IS, Solomon AK, Moore FD. The measurement of total body water in the human subject by deuterium oxide dilution. *J Clin Invest* 1950; 29: 1296-1310.

52. Bankhead MM, Toto RD, Star RA. Accuracy of urea removal estimated by kinetic models. *Kidney Int* 1995; 48: 785-793.

53. Ilstrup K, Hanson G, Shapiro W, Keshaviah P. Examining the foundations of urea kinetics. *Trans Am Soc Artif Intern Organs* 1985; 31: 164-168.

54. Alquist M, Thysell H, Ungerstedt U, Hegbrant J. Development of a urea concentration gradient between muscle interstitium and plasma during hemodialysis. *Int J Artif Organs* 1999; 22: 811-815.

55. Sargent J, Gotch F, Borah M, Piercy L, Spinozzi N, Schoenfeld P, Humphreys M. Urea kinetics: a guide to nutritional management of renal failure. *Am J Clin Nutr* 1978; 31: 1696-1702.

56. Zakaria ER, Lofthouse J, Flessner MF. Effect of intraperitoneal pressures on tissue water of the abdominal muscle. *Am J Physiol Renal Physiol* 2000; 278: F875-885.

57. Odar-Cederlof I, Eriksson CG, Albertioni F, Ericsson F, Kjellstrand CM. Assessment of total body water in hemodialysis patients by a single oral dose of antipyrine. *ASAIO J* 1996; 42: 271-274.

58. Soberman R, Brodie BB, Levy BB, Axelrod J, Hollander V, Steele JM. The use of antipyrine in the measurement of total body water in man. *J Biol Chem* 1949; 179: 31-42.

59. Marshall EK, Emerson K, Cutting WC. The distribution of sulfanilamide in the organism. *J Pharmacol Exp Ther* 1937; 61: 196-204.

60. Painter EE. Total body water in the dog. *Am J Physiol* 1940; 129: 744-755.

61. Danowski TS. Use of thiourea as a measure of change in body water. *J Biol Chem* 1944; 152: 207-212.

62. Sise HS. Distribution of sulfanilamide and acetylsulfanilamide between cells and extracellular fluid. *Proc Soc Exp Biol Med* 1939; 40: 451-454.

63. Chesley LC. Observations on the absorption, apparent volume of distribution, and excretion of thiourea. *J Clin Invest* 1944; 23: 856-858.

64. Williams RH, Kay GA. Absorption, distribution and excretion of thiourea. *Am J Physiol* 1945; 143: 715-722.

65. Grüner O. Bestimmung des Körperwassergahaltes mit hilfe von Alkohol. *Klin Wochenschr* 1957; 35: 347-351.

66. Hoffer EC, Meador CK, Simpson DC. Correlation of whole-body impedance with total body water volume. *J Appl Physiol* 1969; 27: 531-534.

67. Baumgartner RN: Electrical impedance and total body electrical conductivity, in Roche AF, Heymsfield SB, Lohman TG (eds): *Human Body Composition.* Champaign, IL, Human Kinetics, 1996, 79-107.

68. Kushner RF, Schoeller DA, Fjeld CR, Danford L. Is the impedance index (ht^2/R) significant in predicting total body water? *Am J Clin Nutr* 1992; 56: 835-839.

69. De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. *J Appl Physiol* 1997; 82: 1542-1558.

70. Ellis KJ, Wong WW. Human hydrometry: comparison of multifrequency bioelectrical impedance with ^2H_2O and bromine dilution. *J Appl Physiol* 1998; 85: 1056-1062.

71. Hannan WJ, Cowen SJ, Plester CE, Fearon KC, deBeau A. Comparison of bio-impedance spectroscopy and multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients. *Clin Sci* 1995; 89: 651-658.

72. Chioléro RL, Gay LJ, Cotting J, Gurtner C, Schutz Y. Assessment of changes in body water by bioimpedance in acutely ill surgical patients. *Intensive Care Med* 1992; 18: 322-326.

73. Than N, Woodrow G, Oldroyd B, Gonzalez C, Turney JH, Brownjohn AM. Effect of peritoneal fluid on whole body and segmental multiple frequency bioelectrical impedance in patients on peritoneal dialysis. *Eur J Clin Nutr* 2000; 54: 450-451.

74. Cai Y, Jenstrup M, Ide K, Perko M, Secher NH. Influence of temperature on the distribution of blood in humans as assessed by electrical impedance. *Eur J Appl Physiol* 2000; 81: 443-448.

75. Di Iorio BR, Terracciano V, Bellizzi V. Bioelectrical impedance measurement: errors and artifacts. *J Ren Nutr* 1999; 9: 192-197.

76. Watson PE, Watson ID, Batt RD. Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr* 1980; 33: 27-39.

77. Lozano-Nieto A. Human body composition determination: analysis of methods and techniques. *J Clin Eng* 1998; 23: 416-422.

78. Chertow GM, Lazarus JM, Lew NL, Ma L, Lowrie EG. Development of a population-specific regression equation to estimate total body water in hemodialysis patients. *Kidney Int* 1997; 51: 1578-1582.

79. Behnke AR, Feen BG, Wellham WC. The specific gravity of healthy men. Body weight and volume as an index of obesity. *J Am Med Assoc* 1942; 118: 495-498.

80. Siri WE: Body composition from fluid spaces and density: Analysis of methods, in Brozek J, Henschel A (eds): *Techniques for measuring body compostition.* Washington, DC, National Academy of Sciences, National Research Council, 1961, 223-224.

81. Lohman TG. Applicability of body composition techniques and constants for children and youths. *Exerc Sport Sci Rev* 1986; 14: 325-357.

82. Deurenberg P, Weststrate JA, van der Kooy K. Is an adaptation of Siri's formula for the calculation of body fat percentage from body density in the elderly necessary? *Eur J Clin Nutr* 1989; 43: 559-567.

83. Going SB: Densitometry, in Roche AF, Heymsfield SB, Lohman TG (eds): *Human body composition.* Champaign, IL, Human Kinetics, 1996

84. Dempster P, Aitkens S. A new air displacement method for the determination of human body composition. *Med Sci Sports Exerc* 1995; 27: 1692-1697.

85. McCrory MA, Gomez TD, Bernauer EM, Mole PA. Evaluation of a new air displacement plethysmograph for measuring human body composition. *Med Sci Sports Exerc* 1995; 27: 1686-1691.

86. DeVita MV, Stall SH. Dual-energy X-ray absorptiometry: a review. *J Ren Nutr* 1999; 9: 178-181.

87. Roubenoff R, Kehayias JJ, Dawson-Hughes B, Heymsfield SB. Use of dual-energy x-ray absorptiometry in body-composition studies: not yet a "gold standard". *Am J Clin Nutr* 1993; 58: 589-591.

88. Elowsson P, Forslund AH, Mallmin H, Feuk U, Hansson I, Carlsten J. An evaluation of dual-energy X-Ray absorptiometry and underwater weighing to estimate body composition by means of carcass analysis in piglets. *J Nutr* 1998; 128: 1543-1549.

89. Forbes GB: *Human body composition*. New York, Springer-Verlag, 1987.

90. Boling EA, Taylor WL, Entenman C, Behnke AR. Total exchangeable potassium and chloride, and total body water in healthy men of varying fat content. *J Clin Invest* 1962; 41: 1840-1849.

91. Lukaski HC, Mendez J, Buskirk ER, Cohn SH. A comparison of methods of assessment of body composition including neutron activation analysis of total body nitrogen. *Metabolism* 1981; 30: 777-782.

92. Sjöström L, Kvist H, Cederblad A, Tylen U. Determination of total adipose tissue and body fat in women by computed tomography, ^{40}K, and tritium. *Am J Physiol* 1986; 250: E736-745.

93. Sjöström L, Kvist H. Regional body fat measurements with CT-scan and evaluation of anthropometric predictions. *Acta Med Scand Suppl* 1988; 723: 169-177.

94. Kvist H, Chowdhury B, Sjöström L, Tylen U, Cederblad A. Adipose tissue volume determination in males by computed tomography and 40K. *Int J Obes* 1988; 12: 249-266.

95. Ross R, Leger L, Morris D, de Guise J, Guardo R. Quantification of adipose tissue by MRI: relationship with anthropometric variables. *J Appl Physiol* 1992; 72: 787-795.

96. Lewis DS, Rollwitz WL, Bertrand HA, Masoro EJ. Use of NMR for measurement of total body water and estimation of body fat. *J Appl Physiol* 1986; 60: 836-840.

97. Anderson J, Osborn SB, Newton D. Neutron activation analysis in man in vivo. A new technique in medical investigation. *Lancet* 1964; 1201-1205.

98. Cohn SH, Parr RM. Nuclear-based techniques for the in vivo study of human body composition. Report of an Advisory Group of the International Atomic Energy Agency. *Clin Phys Physiol Meas* 1985; 6: 275-301.

99. Beddoe AH, Streat SJ, Hill GL. Evaluation of an in vivo prompt gamma neutron activation facility for body composition studies in critically ill intensive care patients: results on 41 normals. *Metabolism* 1984; 33: 270-280.

100. Hill GL, McCarthy ID, Collins JP, Smith AH. A new method for the rapid measurement of body composition in critically ill surgical patients. *Br J Surg* 1978; 65: 732-735.

101. Heyward VH, Cook KL, Hicks VL, Jenkins KA, Quatrochi JA, Wilson WL. Predictive accuracy of three field methods for estimating relative body fatness of nonobese and obese women. *Int J Sport Nutr* 1992; 2: 75-86.

102. Panotopoulos G, Ruiz JC, Guy-Grand B, Basdevant A. Dual x-ray absorptiometry, bioelectrical impedance, and near infrared interactance in obese women. *Med Sci Sports Exerc* 2001; 33: 665-670.

103. Jones AW. Determination of liquid/air partition coefficients for dilute solutions of ethanol in water, whole blood, and plasma. *J Anal Toxicol* 1983; 7: 193-197.

104. Kalant H: Pharmacokinetics of ethanol: absorption, distribution, and elimination, in Begleiter H, Kissin B (eds): *The pharmacology of alcohol and alcohol dependence*. New York - Oxford, Oxford University Press, 1996, 15-58.

105. Dubowski KM. The blood/breath ratio of ethanol. *Clin Chem* 1979; 25: 1144.

106. Emerson VJ, Holleyhead R, Isaacs MD, Fuller NA, Hunt DJ. The measurement of breath alcohol. The laboratory evaluation of substantive breath test equipment and the report of an operational police trial. *J Forensic Sci Soc* 1980; 20: 3-70.

107. Gullberg RG. Statistical evaluation and reporting of blood alcohol/breath ratio distribution data. *J Anal Toxicol* 1991; 15: 343-344.

108. Labianca DA, Simpson G. Statistical analysis of blood- to breath-alcohol ratio data in the logarithm-transformed and non-transformed modes. *Eur J Clin Chem Clin Biochem* 1996; 34: 111-117.

109. Jones AW, Andersson L. Variability of the blood/breath alcohol ratio in drinking drivers. *J Forensic Sci* 1996; 41: 916-921.

110. Alobaidi TA, Hill DW, Payne JP. Significance of variations in blood:breath partition coefficient of alcohol. *Br Med J* 1976; 2: 1479-1481.

111. Jones AW. Variability of the blood:breath alcohol ratio in vivo. *J Stud Alcohol* 1978; 39: 1931-1939.

112. Dubowski KM. Absorption, distribution and elimination of alcohol: highway safety aspects. *J Stud Alcohol Suppl* 1985; 10: 98-108.

113. Heifer U. Atemalkoholkonzentration/Blutalkoholkonzentration: Utopie eines forensisch brauchbaren Beweismittels. *Blutalkohol* 1986; 23: 229-238.

114. Moore R. Concerning breath alcohol measurements during absorption and elimination. *J Anal Toxicol* 1991; 15: 346-347.

115. Hlastala MP. The alcohol breath test--a review. *J Appl Physiol* 1998; 84: 401-408.

116. George SC, Babb AL, Hlastala MP. Dynamics of soluble gas exchange in the airways. III. Single-exhalation breathing maneuver. *J Appl Physiol* 1993; 75: 2439-2449.

117. Leo A, Hansch C, Elkins D. Partition coefficients and their uses. *Chem Rev* 1971; 71: 525-615.

118. Shusterman AJ, Johnson AS. The role of hydrophobicity and electronic factors in regulating alcohol inhibition of cytochrome P-450-mediated aniline hydroxylation. *Chem Biol Interact* 1990; 74: 63-77.

119. McCreery MJ, Hunt WA. Physico-chemical correlates of alcohol intoxication. *Neuropharmacology* 1978; 17: 451-461.

120. Fang Z, Ionescu P, Chortkoff BS, Kandel L, Sonner J, Laster MJ, Eger EI, 2nd. Anesthetic potencies of n-alkanols: results of additivity and solubility studies suggest a mechanism of action similar to that for conventional inhaled anesthetics. *Anesth Analg* 1997; 84: 1042-1048.

121. Logan BK, Jones AW. Endogenous ethanol 'auto-brewery syndrome' as a drunk-driving defence challenge. *Med Sci Law* 2000; 40: 206-215.

122. Moriya F, Ishizu H. Can microorganisms produce alcohol in body cavities of a living person?: a case report. *J Forensic Sci* 1994; 39: 883-888.

123. Kaji H, Asanuma Y, Yahara O, Shibue H, Hisamura M, Saito N, Kawakami Y, Murao M. Intragastrointestinal alcohol fermentation syndrome: report of two cases and review of the literature. *J Forensic Sci Soc* 1984; 24: 461-471.

124. Widmark EMP. Eine Mikromethode zur Bestimmung von Ätylalkohol im Blut. *Biochem Z* 1922; 131: 473-484.

125. Widmark EMP: *Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung*. Berlin, Urban & Schwarzenberg, 1932.

126. Bonnichsen RK, Wassén A. Crystaline alcohol dehydrogenase from horse liver. *Arch Biochem* 1948; 18: 361-363.

127. Bonnichsen RK, Theorell H. An enzymatic method for the microdetermination of ethanol. *Scand J Clin Lab Invest* 1951; 3: 58-62.

128. Lundquist F, Wolthers H. The kinetics of alcohol elimination in man. *Acta Pharmacol Toxicol* 1958; 14: 265-289.

129. Jones AW, Schuberth J. Computer-aided headspace gas chromatography applied to blood-alcohol analysis: importance of online process control. *J Forensic Sci* 1989; 34: 1116-1127.

130. Cooke AR, Birchall A. Absorption of ethanol from the stomach. *Gastroenterology* 1969; 57: 269-272.

131. Wilkinson PK, Sedman AJ, Sakmar E, Kay DR, Wagner JG. Pharmacokinetics of ethanol after oral administration in the fasting state. *J Pharmacokinet Biopharm* 1977; 5: 207-224.

132. Fraser AG, Rosalki SB, Gamble GD, Pounder RE. Inter-individual and intra-individual variability of ethanol concentration-time profiles: comparison of ethanol ingestion before or after an evening meal. *Br J Clin Pharmacol* 1995; 40: 387-392.

133. Jones AW, Jönsson KÅ, Kechagias S. Effect of high-fat, high-protein, and high-carbohydrate meals on the pharmacokinetics of a small dose of ethanol. *Br J Clin Pharmacol* 1997; 44: 521-526.

134. Shultz J, Weiner H, Westcott J. Retardation of ethanol absorption by food in the stomach. *J Stud Alcohol* 1980; 41: 861-870.

135. Johnson RD, Horowitz M, Maddox AF, Wishart JM, Shearman DJ. Cigarette smoking and rate of gastric emptying: effect on alcohol absorption. *Br Med J* 1991; 302: 20-23.

136. Kechagias S, Jönsson KÅ, Jones AW. Impact of gastric emptying on the pharmacokinetics of ethanol as influenced by cisapride. *Br J Clin Pharmacol* 1999; 48: 728-732.

137. Kechagias S, Jönsson KÅ, Norlander B, Carlsson B, Jones AW. Low-dose aspirin decreases blood alcohol concentrations by delaying gastric emptying. *Eur J Clin Pharmacol* 1997; 53: 241-246.

138. Oneta CM, Simanowski UA, Martinez M, Allali-Hassani A, Pares X, Homann N, Conradt C, Waldherr R, Fiehn W, Coutelle C, Seitz HK. First pass metabolism of ethanol is strikingly influenced by the speed of gastric emptying. *Gut* 1998; 43: 612-619.

139. Amir I, Anwar N, Baraona E, Lieber CS. Ranitidine increases the bioavailability of imbibed alcohol by accelerating gastric emptying. *Life Sci* 1996; 58: 511-518.

140. Roine RP, Gentry RT, Lim RT, Jr., Helkkonen E, Salaspuro M, Lieber CS. Comparison of blood alcohol concentrations after beer and whiskey. *Alcohol Clin Exp Res* 1993; 17: 709-711.

141. Horowitz M, Maddox A, Bochner M, Wishart J, Bratasiuk R, Collins P, Shearman D. Relationships between gastric emptying of solid and caloric liquid meals and alcohol absorption. *Am J Physiol* 1989; 257: G291-298.

142. Näslund E, Bogefors J, Gryback P, Jacobsson H, Hellström PM. Gastric emptying: comparison of scintigraphic, polyethylene glycol dilution, and paracetamol tracer assessment techniques. *Scand J Gastroenterol* 2000; 35: 375-379.

143. Martin E, Moll W, Schmid P, Dettli L. The pharmacokinetics of alcohol in human breath, venous and arterial blood after oral ingestion. *Eur J Clin Pharmacol* 1984; 26: 619-626.

144. Pieters JE, Wedel M, Schaafsma G. Parameter estimation in a three-compartment model for blood alcohol curves. *Alcohol Alcohol* 1990; 25: 17-24.

145. Winne D, Ochsenfahrt H. Die formale Kinetik der Resorption unter Berücksichtigung der Darmdurchblutung. *J Theor Biol* 1967; 14: 293-315.

146. Winne D, Remischovsky J. Intestinal blood flow and absorption of non-dissociable substances. *J Pharm Pharmacol* 1970; 22: 640-641.

147. Levitt MD, Levitt DG. The critical role of the rate of ethanol absorption in the interpretation of studies purporting to demonstrate gastric metabolism of ethanol. *J Pharmacol Exp Ther* 1994; 269: 297-304.

148. Caballeria J, Baraona E, Rodamilans M, Lieber CS. Effects of cimetidine on gastric alcohol dehydrogenase activity and blood ethanol levels. *Gastroenterology* 1989; 96: 388-392.

149. Fraser AG, Hudson M, Sawyerr AM, Smith M, Rosalki SB, Pounder RE. Ranitidine, cimetidine, famotidine have no effect on post-prandial absorption of ethanol 0.8 g/kg taken after an evening meal. *Aliment Pharmacol Ther* 1992; 6: 693-700.

150. Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. *N Engl J Med* 1990; 322: 95-99.

151. Gentry RT, Baraona E, Lieber CS. Agonist: gastric first pass metabolism of alcohol. *J Lab Clin Med* 1994; 123: 21-26; discussion 27.

152. Roine R, Gentry RT, Hernandez-Muñoz R, Baraona E, Lieber CS. Aspirin increases blood alcohol concentrations in humans after ingestion of ethanol. *JAMA* 1990; 264: 2406-2408.

153. Haber PS, Gentry RT, Mak KM, Mirmiran-Yazdy SA, Greenstein RJ, Lieber CS. Metabolism of alcohol by human gastric cells: relation to first-pass metabolism. *Gastroenterology* 1996; 111: 863-870.

154. Gentry RT, Baraona E, Amir I, Roine R, Chayes ZW, Sharma R, Lieber CS. Mechanism of the aspirin-induced rise in blood alcohol levels. *Life Sci* 1999; 65: 2505-2512.

155. Baraona E, Abittan CS, Dohmen K, Moretti M, Pozzato G, Chayes ZW, Schaefer C, Lieber CS. Gender differences in pharmacokinetics of alcohol. *Alcohol Clin Exp Res* 2001; 25: 502-507.

156. Levitt MD, Li R, DeMaster EG, Elson M, Furne J, Levitt DG. Use of measurements of ethanol absorption from stomach and intestine to assess human ethanol metabolism. *Am J Physiol* 1997; 273: G951-957.

157. Levitt MD, Levitt DG. Appropriate use and misuse of blood concentration measurements to quantitate first-pass metabolism. *J Lab Clin Med* 2000; 136: 275-280.

158. Pastino GM, Conolly RB. Application of a physiologically based pharmacokinetic model to estimate the bioavailability of ethanol in male rats: distinction between gastric and hepatic pathways of metabolic clearance. *Toxicol Sci* 2000; 55: 256-265.

159. Gentry RT. Determinants and analysis of blood alcohol concentrations after social drinking. *Alcohol Clin Exp Res* 2000; 24: 399.

160. Grant BF. The relationship between ethanol intake and DSM-III-R alcohol dependence: results of a national survey. *J Subst Abuse* 1993; 5: 257-267.

161. Dawson DA, Archer LD, Grant BF. Reducing alcohol-use disorders via decreased consumption: a comparison of population and high-risk strategies. *Drug Alcohol Depend* 1996; 42: 39-47.

162. York JL, Hirsch JA. Association between blood pressure and lifetime drinking patterns in moderate drinkers. *J Stud Alcohol* 1997; 58: 480-485.

163. Devgun MS, Dunbar JA. Alcohol consumption, blood alcohol level and the relevance of body weight in experimental design and analysis. *J Stud Alcohol* 1990; 51: 24-28.

164. Mirand AL, Welte JW. Total body water adjustment of mean alcohol intakes. *J Subst Abuse* 1994; 6: 419-425.

165. Graham K, Wilsnack R, Dawson D, Vogeltanz N. Should alcohol consumption measures be adjusted for gender differences? *Addiction* 1998; 93: 1137-1147.

166. Dundee JW, Knox JW, Isaac M. Blood, urine, and breath levels after rapid intravenous infusion of ethanol. *Br Med J* 1970; 3: 552-554.

167. Dundee JW, Isaac M, Taggart J. Blood ethanol levels following rapid intravenous infusion. *Q J Stud Alcohol* 1971; 32: 741-747.

168. Isaac M, Pandit SK, Dundee JW, Galway JE. Intravenous ethanol anaesthesia. A study of sequelae and their implications. *Acta Anaesthesiol Scand* 1971; 15: 141-155.

169. Rangno RE, Kreeft JH, Sitar DS. Ethanol 'dose-dependent' elimination: Michaelis-Menten v classical kinetic analysis. *Br J Clin Pharmacol* 1981; 12: 667-673.

170. Derr RF. Simulation studies on ethanol metabolism in different human populations with a physiological pharmacokinetic model. *J Pharm Sci* 1993; 82: 677-682.

171. Crabb DW, Dipple KM, Thomasson HR. Alcohol sensitivity, alcohol metabolism, risk of alcoholism, and the role of alcohol and aldehyde dehydrogenase genotypes. *J Lab Clin Med* 1993; 122: 234-240.

172. Bosron WF, Ehrig T, Li TK. Genetic factors in alcohol metabolism and alcoholism. *Semin Liver Dis* 1993; 13: 126-135.

173. Li TK, Yin SJ, Crabb DW, O'Connor S, Ramchandani VA. Genetic and environmental influences on alcohol metabolism in humans. *Alcohol Clin Exp Res* 2001; 25: 136-144.

174. Yoshida A, Rzhetsky A, Hsu LC, Chang C. Human aldehyde dehydrogenase gene family. *Eur J Biochem* 1998; 251: 549-557.

175. Peng GS, Wang MF, Chen CY, Luu SU, Chou HC, Li TK, Yin SJ. Involvement of acetaldehyde for full protection against alcoholism by homozygosity of the variant allele of mitochondrial aldehyde dehydrogenase gene in Asians. *Pharmacogenetics* 1999; 9: 463-476.

176. Iwatsubo T, Hirota N, Ooie T, Suzuki H, Sugiyama Y. Prediction of in vivo drug disposition from in vitro data based on physiological pharmacokinetics. *Biopharm Drug Dispos* 1996; 17: 273-310.

177. Gabrielsson J, Weiner D: *Pharmacokinetic and pharmacodynamic data analysis: Concepts and applications*, (3rd ed). Stockholm, Swedish Pharmaceutical Press, 2000.

178. Keiding S, Johansen S, Midtboll I, Rabol A, Christiansen L. Ethanol elimination kinetics in human liver and pig liver in vivo. *Am J Physiol* 1979; 237: E316-324.

179. Dedrick RL, Forrester DD. Blood flow limitations in interpreting Michaelis constants for ethanol oxidation in vivo. *Biochem Pharmacol* 1973; 22: 1133-1140.

180. Greenway CV. Effects of hepatic blood flow on hepatic ethanol kinetics measured in cats and predicted from the parallel tube model. *Can J Physiol Pharmacol* 1989; 67: 728-733.

181. Tsutsumi M, Lasker JM, Takahashi T, Lieber CS. In vivo induction of hepatic P4502E1 by ethanol: role of increased enzyme synthesis. *Arch Biochem Biophys* 1993; 304: 209-218.

182. Wang PY, Kaneko T, Tsukada H, Sato A. Dose and route dependency of metabolism and toxicity of chloroform in ethanol-treated rats. *Arch Toxicol* 1994; 69: 18-23.

183. Fuhr U. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet* 2000; 38: 493-504.

184. Asai H, Imaoka S, Kuroki T, Monna T, Funae Y. Microsomal ethanol oxidizing system activity by human hepatic cytochrome P450s. *J Pharmacol Exp Ther* 1996; 277: 1004-1009.

185. Fujimiya T, Yamaoka K, Fukui Y. Parallel first-order and Michaelis-Menten elimination kinetics of ethanol. Respective role of alcohol dehydrogenase (ADH), non-ADH and first-order pathways. *J Pharmacol Exp Ther* 1989; 249: 311-317.

186. Shoaf SE. Pharmacokinetics of intravenous alcohol: two compartment, dual Michaelis-Menten elimination. *Alcohol Clin Exp Res* 2000; 24: 424-425.

187. Jones AW: Biochemistry and physiology of alcohol: applications to forensic science and toxicology, in Garriott JC (ed): *Medicolegal aspects of alcohol*. Tucson, Lawyers and Judges Publishing Company Inc, 1996, 85-136.

188. Thomasson HR. Gender differences in alcohol metabolism. Physiological responses to ethanol. *Recent Dev Alcohol* 1995; 12: 163-179.

189. Mumenthaler MS, Taylor JL, O'Hara R, Yesavage JA. Gender differences in moderate drinking effects. *Alcohol Res Health* 1999; 23: 55-64.

190. Jones AW, Neri A. Age-related differences in blood ethanol parameters and subjective feelings of intoxication in healthy men. *Alcohol Alcohol* 1985; 20: 45-52.

191. Vestal RE, McGuire EA, Tobin JD, Andres R, Norris AH, Mezey E. Aging and ethanol metabolism. *Clin Pharmacol Ther* 1977; 21: 343-354.

192. Li TK, Beard JD, Orr WE, Kwo PY, Ramchandani VA, Thomasson HR. Variation in ethanol pharmacokinetics and perceived gender and ethnic differences in alcohol elimination. *Alcohol Clin Exp Res* 2000; 24: 415-416.

193. Li TK, Beard JD, Orr WE, Kwo PY, Ramchandani VA, Thomasson HR. Genetic and ethnic differences in alcohol metabolism. Abstract. *Alcohol Clin Exp Res* 1998; 22: 771-772.

194. Lieber CS. Ethnic and gender differences in ethanol metabolism. *Alcohol Clin Exp Res* 2000; 24: 417-418.

195. Minors DS, Waterhouse JM. Ethanol pharmacokinetics in healthy man: Michaelis-Menten parameters and the circadian rhythm. *Chronobiologia* 1985; 12: 137-144.

196. Sharma R, Gentry RT, Lim RT, Jr., Lieber CS. First-pass metabolism of alcohol. Absence of diurnal variation and its inhibition by cimetidine after evening meal. *Dig Dis Sci* 1995; 40: 2091-2097.

197. Keiding S, Christensen NJ, Damgaard SE, Dejgard A, Iversen HL, Jacobsen A, Johansen S, Lundquist F, Rubinstein E, Winkler K. Ethanol metabolism in heavy drinkers after massive and moderate alcohol intake. *Biochem Pharmacol* 1983; 32: 3097-3102.

198. Lumeng L, Bosron WF, Li TK. Quantitative correlation of ethanol elimination rates in vivo with liver alcohol dehydrogenase activities in fed, fasted and food-restricted rats. *Biochem Pharmacol* 1979; 28: 1547-1551.

199. Kwo PY, Ramchandani VA, O'Connor S, Amann D, Carr LG, Sandrasegaran K, Kopecky KK, Li TK. Gender differences in alcohol metabolism: relationship to liver volume and effect of adjusting for body mass. *Gastroenterology* 1998; 115: 1552-1557.

200. Loeppky JA, Myhre LG, Venters MD, Luft UC. Total body water and lean body mass estimated by ethanol dilution. *J Appl Physiol* 1977; 42: 803-808.

201. Walle AJ, Grüner O, Niedermayer W. Measurement of total body water in patients on maintenance hemodialysis using an ethanol dilution technique. *Nephron* 1980; 26: 286-290.

202. Tzamaloukas AH, Jackson JE, Gallegos JC, Long DA, McLane MM. Distribution volume of ethanol as a measure of body water. *Miner Electrolyte Metab* 1985; 11: 123-130.

203. Endres HG, Grüner O. Comparison of D_2O and ethanol dilutions in total body water measurements in humans. *Clin Investig* 1994; 72: 830-837.

204. Dahl NV, Foote EF, Kapoian T, Steward CA, Sherman RA. Measuring total body water in peritoneal dialysis patients using an ethanol dilution technique. *Kidney Int* 1999; 56: 2297-2303.

205. Pawan GLS, Hoult WH. The determination of total body water in man with ethanol dilution. *Proc Biochem Soc* 1963; 87: 6P.

206. Avadhany ST, Shetty PS. Determination of total body water in vivo by ethanol dilution in man. *Indian J Med Res* 1986; 84: 217-222.

207. Grüner O, Salmen A. Vergleichende Körperwasserbestimmungen mit Hilfe von N-Acetyl-4-aminoantipyrin und Alkohol. *Klin Wochenschr* 1961; 39: 92-97.

208. Jones AW, Hahn RG, Stalberg HP. Pharmacokinetics of ethanol in plasma and whole blood: estimation of total body water by the dilution principle. *Eur J Clin Pharmacol* 1992; 42: 445-448.

209. Grüner O, Endres HG. Die Alkoholmethode zur Bestimmung des Gesamtkörperwassers: Vergleich mit Schwerem Wasser (D2O). *Wien Med Wochenschr* 1993; 143: 106-110.

210. Hodgson BT, Shajani NK. Distribution of ethanol: plasma to whole blood ratios. *Can Soc Forensic Sci J* 1985; 18: 73-77.

211. Winek CL, Carfagna M. Comparison of plasma, serum, and whole blood ethanol concentrations. *J Anal Toxicol* 1987; 11: 267-268.

212. Jones AW, Hahn RG, Stalberg HP. Distribution of ethanol and water between plasma and whole blood; inter- and intra-individual variations after administration of ethanol by intravenous infusion. *Scand J Clin Lab Invest* 1990; 50: 775-780.

213. Watson PE, Watson ID, Batt RD. Prediction of blood alcohol concentrations in human subjects. Updating the Widmark Equation. *J Stud Alcohol* 1981; 42: 547-556.

214. Slogoff S, Keats AS, Arlund C. On the safety of radial artery cannulation. *Anesthesiology* 1983; 59: 42-47.

215. Durbin CG, Jr. Radial arterial lines and sticks: what are the risks? *Respir Care* 2001; 46: 229-231.

216. Klein PD, Klein ER. Stable isotopes: origins and safety. *J Clin Pharmacol* 1986; 26: 378-382.

217. Goldberg L. Quantitative studies on alcohol tolerance in man. *Acta Physiol Scand* 1943; 5, suppl 16: 1-128.

218. Mellanby E: *Alcohol: Its absorption into and disappearance from from the blood under different conditions* Medical Research Committee Special Report No. 31. London, HMSO, 1919, 1-48.

219. Bramley PN, Heighton RW, Green JH. Methodological considerations in arterialization of venous blood. *Clin Chem* 1991; 37: 1139-1140.

220. Godsland IF, Felton C, Wynn V. Venous blood arterialization and parameters from the minimal models of glucose disappearance and post-hepatic insulin delivery. *Clin Sci* 1993; 85: 89-95.

221. Green JH, Ellis FR, Shallcross TM, Bramley PN. Invalidity of hand heating as a method to arterialize venous blood. *Clin Chem* 1990; 36: 719-722.

222. de Marchi SF, Schwerzmann M, Billinger M, Windecker S, Meier B, Seiler C. Sympathetic stimulation using the cold pressor test increases coronary collateral flow. *Swiss Med Wkly* 2001; 131: 351-356.

223. Van Slyke DD, Phillips RA, Dole VP, Hamilton PB, Archibald RM, Plazin J. Calculation of hemoglobin from blood specific gravities. *J Biol Chem* 1950; 183: 349-360.

224. Ashworth CT, Adams G. Blood specific gravity studies. *J Lab Clin Med* 1941; 26: 1934-1939.

225. Trudnowski RJ, Rico RC. Specific gravity of blood and plasma at 4 and 37 degrees C. *Clin Chem* 1974; 20: 615-616.

226. Kühnholz B, Kaatsch HJ, Thomsen H, Nguyen D. Zur Dauer der Resorptionszeit bei einer Ethanolbelastung von 0.3, 0.5 und 0.8 g Alkohol pro kg Körpergewicht in 30 Minuten. *Blutalkohol* 1993; 30: 158-165.

227. Ammon E, Schafer C, Hofmann U, Klotz U. Disposition and first-pass metabolism of ethanol in humans: is it gastric or hepatic and does it depend on gender? *Clin Pharmacol Ther* 1996; 59: 503-513.

228. Wilkinson PK, Sedman AJ, Sakmar E, Earhart RH, Weidler DJ, Wagner JG. Blood ethanol concentrations during and following constant-rate intravenous infusion of alcohol. *Clin Pharmacol Ther* 1976; 19: 213-223.

229. Gibaldi M, Perrier D: *Pharmacokinetics*, (2nd ed). New York, M. Dekker, 1982.

230. Bookbinder MJ, Panosian KJ. Correct and incorrect estimation of within-day and between-day variation. *Clin Chem* 1986; 32: 1734-1737.

231. Rodbard D. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clin Chem* 1974; 20: 1255-1270.

232. Wagner JG. Inter- and intrasubject variation of digoxin renal clearance in normal adult males. *Drug Intell Clin Pharm* 1988; 22: 562-567.

233. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307-310.

234. Rowland M, Tozer TN: *Clinical Pharmacokinetics, Concepts and Applications*, (3rd ed). Media, PA, Williams & Wilkins, 1995.

235. Wagner JG, Wilkinson PK, Sedman AJ, Kay DR, Weidler DJ. Elimination of alcohol from human blood. *J Pharm Sci* 1976; 65: 152-154.

236. Mumenthaler MS, Taylor JL, Yesavage JA. Ethanol pharmacokinetics in white women: nonlinear model fitting versus zero-order elimination analyses. *Alcohol Clin Exp Res* 2000; 24: 1353-1362.

237. Wagner JG, Wilkinson PK, Ganes DA. Parameters Vm' and Km for elimination of alcohol in young male subjects following low doses of alcohol. *Alcohol Alcohol* 1989; 24: 555-564.

238. Kohlenberg-Müller K, Bitsch I. Neue Methoden zur pharmakokinetischen Beschreibung des Alkohols und seiner Metaboliten bei weiblichen und männlichen Versuchspersonen. *Blutalkohol* 1990; 27: 40-48.

239. Eggleton MG. The diuretic action of alcohol in man. *J Physiol* 1942; 101: 172-191.

240. Rubini ME, Kleeman CR, Lamdin E. Studies on alcohol diuresis. I. The effect of ethyl alcohol ingestion on water, electrolyte and acid-base metabolism. *J Clin Invest* 1955; 34: 439-447.

241. Roberts KE. Mechanism of dehydration following alcohol ingestion. *Arch Int Med* 1963; 112: 154-157.

242. Taivainen H, Laitinen K, Tahtela R, Kilanmaa K, Valimaki MJ. Role of plasma vasopressin in changes of water balance accompanying acute alcohol intoxication. *Alcohol Clin Exp Res* 1995; 19: 759-762.

243. Eisenhofer G, Johnson RH. Effect of ethanol ingestion on plasma vasopressin and water balance in humans. *Am J Physiol* 1982; 242: R522-527.

244. Kleeman CR, Rubini ME, Lambin E, Epstein FH. Studies on alcohol diuresis. II. The evaluation of ethyl alcohol as an inhibitor of the neurohypophysis. *J Clin Invest* 1955; 34: 448-455.

245. Hynynen M, Kupari M, Salmenpera M, Koskinen P, Tikkanen I, Fyhrquist F. Plasma atrial natriuretic factor during ethanol ingestion in volume-loaded subjects. *Alcohol Alcohol* 1992; 27: 277-285.

246. Pohorecky LA, Packard K. Noradrenergic mechanisms in ethanol diuresis. *Alcohol Clin Exp Res* 1986; 10: 177-183.

247. Mascord D, Smith J, Starmer GA, Whitfield JB. Effect of oral glucose on the rate of metabolism of ethanol in humans. *Alcohol Alcohol* 1988; 23: 365-370.

248. Svensson CK, Edwards DJ, Mauriello PM, Barde SH, Foster AC, Lanc RA, Middleton E, Jr., Lalka D. Effect of food on hepatic blood flow: implications in the "food effect" phenomenon. *Clin Pharmacol Ther* 1983; 34: 316-323.

249. Larsen JA. Determination of hepatic blood flow by means of ethanol. *Scand J Clin Lab Invest* 1959; 11: 340-347.

250. Greenway CV, Lautt WW, Sitar D. Measurement of hepatic blood flow from ethanol clearance in anesthetized cats. *Hepatology* 1990; 11: 513.

251. Silva G, Fluxá F, Bresky G, Backhouse C, Palma M, Ruiz M, Hirsch S, Iturriaga H. Splanchnic and systemic hemodynamics in early abstinence and after ethanol administration in non-cirrhotic alcoholic patients. *J Hepatol* 1994; 20: 494-499.

252. Bosron WF, Crabb DW, Housinger TA, Li TK. Effect of fasting on the activity and turnover of rat liver alcohol dehydrogenase. *Alcohol Clin Exp Res* 1984; 8: 196-200.

253. Sonnenberg GE, Keller U. Sampling of arterialized heated-hand venous blood as a noninvasive technique for the study of ketone body kinetics in man. *Metabolism* 1982; 31: 1-5.

254. Hahn RG, Jones AW, Norberg Å. Abnormal blood-ethanol profile associated with stress. *Clin Chem* 1992; 38: 1193-1194.

255. Davies BT, Bowen CK. Total body water and peak alcohol concentration: a comparative study of young, middle-age, and older females. *Alcohol Clin Exp Res* 1999; 23: 969-975.

256. Chiou WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part II). *Clin Pharmacokinet* 1989; 17: 275-290.

257. Chiou WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part I). *Clin Pharmacokinet* 1989; 17: 175-199.

258. Harger RN: Blood source and alcohol level: errors from using venous blood during active absorption. Paper presented at the Third international conference on alcohol and road traffic, Havard J (ed), British Medical Association, London, 1963; 212-219.

259. Sedman AJ, Wilkinson PK, Wagner JG. Concentrations of ethanol in two segments of the vascular system. *J Forensic Sci* 1976; 21: 315-322.

260. Herscovitch P, Raichle ME. What is the correct value for the brain-blood partition coefficient for water? *J Cereb Blood Flow Metab* 1985; 5: 65-69.

261. Frackowiak RS, Lenzi GL, Jones T, Heather JD. Quantitative measurement of regional cerebral blood flow and oxygen metabolism in man using ^{15}O and positron emission tomography: theory, procedure, and normal values. *J Comput Assist Tomogr* 1980; 4: 727-736.

262. Lentner C, CIBA-GEIGY Limited.: Vol 1, Units of measurement, body fluids, composition of the body, nutrition, *Geigy scientific tables* (8^{th} ed). Basle, Switzerland, Ciba-Geigy, 1981

263. Kaneko T, Wang PY, Sato A. Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. *Occup Environ Med* 1994; 51: 68-72.

264. Meulenberg CJ, Vijverberg HP. Empirical relations predicting human and rat tissue:air partition coefficients of volatile organic compounds. *Toxicol Appl Pharmacol* 2000; 165: 206-216.

265. Fiserova-Bergerova V, Diaz ML. Determination and prediction of tissue-gas partition coefficients. *Int Arch Occup Environ Health* 1986; 58: 75-87.

266. Schaefer GJ, Richardson WR, Bonsall RW, Michael RP. Brain self-stimulation, locomotor activity and tissue concentrations of ethanol in male rats. *Drug Alcohol Depend* 1988; 21: 67-75.

267. Erickson CK. Regional distribution of ethanol in rat brain. *Life Sci* 1976; 19: 1439-1446.

268. Gill K, France C, Amit Z. Voluntary ethanol consumption in rats: an examination of blood/brain ethanol levels and behavior. *Alcohol Clin Exp Res* 1986; 10: 457-462.

269. Sunahara GI, Kalant H, Schofield M, Grupp L. Regional distribution of ethanol in the rat brain. *Can J Physiol Pharmacol* 1978; 56: 988-992.

270. Moser E, Holzmueller P, Krssak M. Improved estimation of tissue hydration and bound water fraction in rat liver tissue. *Magma* 1996; 4: 55-59.

271. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev* 1993; 73: 1-78.

272. Israelachvili J, Wennerström H. Role of hydration and water structure in biological and colloidal interactions. *Nature* 1996; 379: 219-225.

273. Cameron IL, Kanal KM, Keener CR, Fullerton GD. A mechanistic view of the non-ideal osmotic and motional behavior of intracellular water. *Cell Biol Int* 1997; 21: 99-113.

274. Wiggins PM. High and low density water and resting, active and transformed cells. *Cell Biol Int* 1996; 20: 429-435.

275. Channareddy S, Jose SS, Janes N. Ethanol binding to a model carbohydrate, glycogen. *Biochem Pharmacol* 1996; 52: 1911-1914.

276. Freund G. Possible relationships of alcohol in membranes to cancer. *Cancer Res* 1979; 39: 2899-2901.

277. Klemm WR. Biological water and its role in the effects of alcohol. *Alcohol* 1998; 15: 249-267.

278. Klemm WR. Dehydration: a new alcohol theory. *Alcohol* 1990; 7: 49-59.

279. Pohorecky LA, Brick J. Pharmacology of ethanol. *Pharmacol Ther* 1988; 36: 335-427.

280. Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, Kalant H, Koob GF, Li TK, Tabakoff B. Effects of moderate alcohol consumption on the central nervous system. *Alcohol Clin Exp Res* 1998; 22: 998-1040.

281. Gould L, Reddy CV, Becker W, Oh KC, Kim SG. Electrophysiologic properties of alcohol in man. *J Electrocardiol* 1978; 11: 219-226.

282. Child JS, Kovick RB, Levisman JA, Pearce ML. Cardiac effects of acute ethanol ingestion unmasked by autonomic blockade. *Circulation* 1979; 59: 120-125.