Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline

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Frassetto, Lynda A., R. Curtis Morris, Jr., and Anthony Sebastian. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. *Am. J. Physiol.* 271 (*Renal Fluid Electrolyte Physiol.* 40): F1114–F1122, 1996.—In 64 apparently healthy adult humans (ages 17–74 yr) ingesting controlled diets, we investigated the separate and combined effects of age, glomerular filtration rate (GFR, index of age-related renal functional decline), renal net acid excretion [NAE, index of endogenous acid production (EAP)], and blood P_{CO_2} (P_bCO_2, index of respiratory set point) on steady-state blood hydrogen ion ($[H^+]_b$) and plasma bicarbonate concentration ($[HCO_3^-]_p$). Independent predictors of $[H^+]_b$ and $[HCO_3^-]_p$ were P_bCO_2, NAE, and either age or GFR, but not both, because the two were highly correlated (inversely). $[H^+]_b$ increased with increasing P_bCO_2, NAE, and age and with decreasing GFR. $[HCO_3^-]_p$ decreased with increasing NAE and age but increased with increasing P_bCO_2 and GFR. Age (or GFR) at constant NAE had greater effect on both $[H^+]_b$ and $[HCO_3^-]_p$ than did NAE at constant age (or GFR). Neither P_bCO_2 nor NAE correlated with age or GFR. Thus two metabolic factors, diet-dependent EAP and age (or GFR), operate independently to determine blood acid-base composition in adult humans. Otherwise healthy adults manifest a low-grade diet-dependent metabolic acidosis, the severity of which increases with age at constant EAP, apparently due in part to the normal age-related decline of renal function.

glomerular filtration rate; diet; kidney; bone

IN NORMAL ADULT HUMANS eating ordinary American diets, systemic acid-base equilibrium is maintained within narrow limits (20, 24, 27, 37). That occurs despite a continuing load of net acid to the systemic circulation generated as end products of metabolism of neutral precursors in the diet (e.g., sulfuric acid generated from the metabolism of sulfur-containing amino acids) (20, 22). Stability of systemic acid-base equilibrium is critically dependent on excretion of acid in urine (34), the rate of which the kidney adjusts in keeping with diet-induced variations in the net endogenous acid load. Since renal function progressively decreases with age (8), for a given net endogenous acid load, blood acidity might increase with age, and plasma bicarbonate concentration ($[HCO_3^-]_p$) might decrease. Such metabolic acidosis, though mild, might over time contribute to the pathogenesis of the physiological disturbances and degenerative diseases characteristic of aging. Yet, little attention has been given to the effect of age on systemic acid-base equilibrium in healthy subjects.

In a previous approach to determine whether the acid-base composition of the blood in normal adult humans changes in relation to age, we assembled a database of blood acid-base measurements from multiple individual published reports of normal values in adult subjects whose ages were specified (12). We found that with increasing age, steady-state blood hydrogen ion concentration ($[H^+]_b$) increases ($P < 0.001$) and steady-state $[HCO_3^-]_p$ decreases ($P < 0.001$), indicative of the occurrence of a progressively severe low-grade metabolic acidosis with age. Blood carbon dioxide tension (P_bCO_2) decreased with age ($P < 0.05$), in keeping with the expected respiratory adaptation to increased blood acidity.

For several reasons, however, the results of such a meta-analysis cannot be interpreted conclusively. Because the reviewed reports lacked data on rates of endogenous acid production (EAP), the possibility could not be excluded that the observed age-related changes in acid-base equilibrium reflected predominantly differences in diet-dependent rates of EAP among individuals. Furthermore, since the reviewed reports were published over a span of 50 years, the apparent effect of age might reflect differences in techniques of specimen sampling and processing or in analytical methodology among laboratories. Moreover, only a few reports provided data on renal function.

To circumvent those difficulties, and to investigate the role of the aging kidney in regulating systemic acid-base equilibrium, we measured $[H^+]_b$ and carbon dioxide tension (P_{CO_2}), $[HCO_3^-]_p$, renal net acid excretion (NAE), and glomerular filtration rate (GFR) in 64 healthy adult men and women over a wide range of ages, each of whom was in a steady-state on a controlled diet while residing in a clinical research center.

METHODS

Chronic metabolic balance studies were carried out in 64 normal[1] subjects (39 men, 25 women), whose ages ranged from 17 to 74 yr. The studies were carried out while the patients were hospitalized in the General Clinical Research Center at the University of California, San Francisco (UCSF), between 1979 and 1990. The studies were approved by the UCSF Committee on Human Research, and each subject gave informed consent.

Each subject ingested one of nine diets that yielded EAP rates varying over a wide range (around 25–150 meq/day) within the normal range (see below). Although the nine diets differed in composition, the recipe for each diet was identical for all subjects eating the same diet, both with respect to the

[1] Use of the term "normal" in reference to the subjects studied is intended to imply only that the subjects were free of clinically overt disease as assessed by routine history, physical, and laboratory examination and not that the subjects were free of the pathological conditions that typically occur in aging humans, such as the pathological changes occurring in the aging kidney.

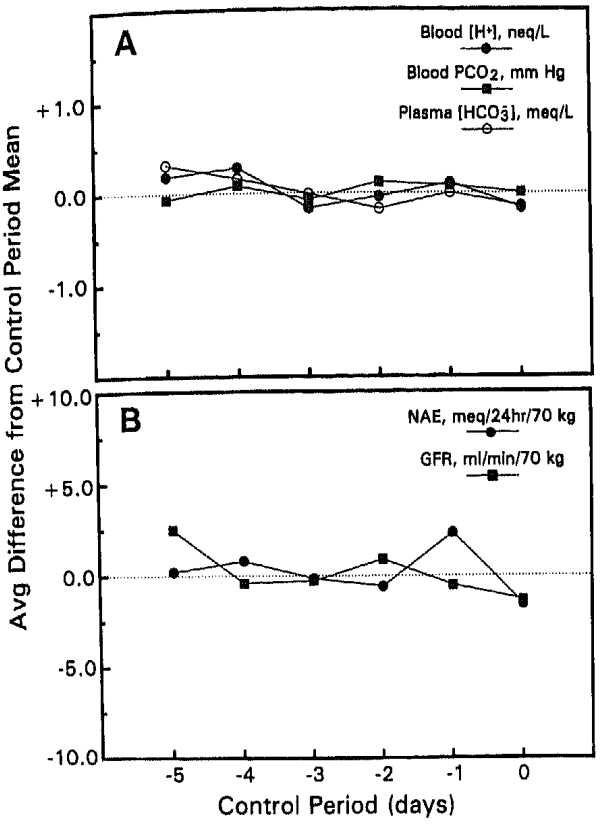

Fig. 1. Demonstration of the steady state during the period of observation for blood hydrogen ion concentration ($[H^+]_b$), plasma bicarbonate concentration ($[HCO_3^-]_p$), and blood carbon dioxide tension (P_bCO_2) (*A*) and glomerular filtration rate, expressed as 24-h creatinine clearance (GFR), and net acid excretion (NAE) (*B*) (n = 64). For each variable is depicted the time course of the daily differences from the mean for the period of observation. Each data point represents the mean of the differences for all subjects. There was no significant trend in these variables during the period of observation.

specific food items (including beverages and free water) making up the diet and the quantity of each item fed to each subject per day per kilogram body weight. Before the collection of specimens was initiated for measurements of plasma and urine acid-base and electrolyte composition, the subjects ingested the diets for an average of 11 days (range 2–25 days) (pre-steady-state period). During the steady-state period that followed (Fig. 1), which averaged 6 days (range 3–9 days), the means of four blood specimens and six urine specimens were obtained from each subject.

The original purpose of the study for each of the nine separate diet groups was not the same. The differences in study purpose are reflected in experimental maneuvers (not described) carried out subsequent to the above-described steady-state period utilized for the present analysis of the effect of age on plasma acid-base composition. The present study thus constitutes a retrospective analysis of control data obtained for different purposes. But, except for the variable of diet, the experimental conditions were identical among subjects prior to and during the steady-state period selected for the present analysis. Of the 64 subjects whose data was analyzed for this study for the effect of age (GFR) on blood acid-base composition, data on 16 subjects were previously analyzed exclusively for the effect of diet acid load on blood acid-base composition (20). The sample size of that previous study was too small to allow assessment of the effect of age and GFR.

With the subjects sitting, arterialized venous blood specimens were obtained without stasis or exposure to air from a superficial vein on the back of the hand that had been heated by immersion in a thermoregulated (43–44°C) water bath for 10–15 min. The specimens were routinely analyzed for pH, P_{CO_2}, total CO_2, sodium, potassium, chloride, and creatinine.

Spontaneously voided urine specimens were maintained under a thin layer of mineral oil and preserved with thymol for determination of pH and CO_2 content. In addition, the following analyses were carried out: total volume; and concentrations of ammonium, titratable acid, sodium, potassium, chloride, inorganic phosphorus, and creatinine.

The pH of blood, oxygen, and P_{CO_2} were measured at 37°C with either a Radiometer ABL-30 or a Radiometer BMS-3 blood-gas analyzer; urine pH was measured at 37°C with a different Radiometer BMS-3 blood-gas analyzer. Plasma and urine total carbon dioxide content were determined either by manometry (using the Natelson microgasometer) or by thermal conductivity (Corning CO_2 analyzer); the two methods have similar precision [Natelson, coefficient of variation (CV) = 1.2%; Ericson, CV = 0.7%] and, within an examined range of values of total carbon dioxide content from 22 to 33 mmol/l, yield nearly identical values [ΔX = 0.1 ± 0.5 mmol/l (±SD), n = 27]. $[HCO_3^-]_p$ and P_{CO_2} are calculated from the measured values of blood pH and plasma carbon dioxide content utilizing the Henderson-Hasselbalch equation, where pK' (6.1, 37°C) is corrected for pH and body temperature (33), and the solubility coefficient of carbon dioxide in plasma (0.0301, 37°C) is corrected for body temperature (33). Blood pH values were also corrected for body temperature (33). Urine bicarbonate concentration was calculated from the measured values of urine pH and carbon dioxide content utilizing the Henderson-Hasselbalch equation, where the solubility coefficient of CO_2 is taken as 0.0309 and pK' is corrected for ionic strength as follows: $pK' = 6.33 - 0.5 \cdot ([Na^+] + [K^+])^{1/2}$, where Na^+ and K^+ concentrations are expressed in equivalents per liter. NAE was calculated as the sum of the excretion rates of titratable acid and ammonium minus that of bicarbonate and is expressed per 70 kg body wt. Titratable acid concentration was determined by titration, and urine ammonium concentration was determined as previously described (35).

NAE is utilized as the indicator of net endogenous noncarbonic acid production, since, under steady-state conditions in normal subjects, there is a predictable linear relationship between these two variables (22). GFR was measured as 24-h creatinine clearance, expressed per 70 kg body wt, and taken as an index of renal functional integrity.

For each subject and each blood and urine variable, the averages of all steady-state measurements were computed, and those were used as the primary database. Statistical analyses were carried out using Sigmastat (Jandel Scientific Software, San Rafael, CA), including simple and multiple linear regression, nonlinear regression, and correlation analyses. Multiple regression analyses included calculation of standardized regression coefficients for comparison of the relative quantitative impact of predictor variables on the dependent variable.

RESULTS

Blood [H⁺]. By simple correlation analysis, among subjects, steady-state blood acidity correlated positively with age (r = +0.40, $P < 0.002$) and P_bCO_2 (r =

Table 1. *Matrix of correlations of steady-state values of acid-base variables in normal subjects*

	P_bCO_2	NAE	Age	GFR
$[H^+]_b$				
r	0.434	0.199	0.397	−0.327
P	<0.001	0.114	<0.002	0.008
$[HCO_3^-]_p$				
r	0.706	−0.320	−0.394	0.455
P	<0.001	0.010	<0.002	<0.001
P_bCO_2				
r		−0.140	−0.073	0.201
P		0.268	0.566	0.111
NAE				
r			−0.048	−0.034
P			0.704	0.798
Age				
r				−0.691
P				<0.001

Vertical numeral pairs indicate correlation coefficient (*r*) and the probability of error (*P*) in rejecting the hypothesis that $r = 0$, respectively. $[H^+]_b$, blood hydrogen ion concentration; $[HCO_3^-]_p$, plasma bicarbonate concentration; P_bCO_2, blood carbon dioxide tension; NAE, net acid excretion; GFR, glomerular filtration rate.

+0.43, $P < 0.001$), negatively with $[HCO_3^-]_p$[2] ($r = -0.33$, $P < 0.01$) and GFR ($r = -0.33$, $P < 0.01$), and not significantly with NAE ($r = +0.20$, $P < 0.2$) (Table 1; Figs. 2 and 3). GFR correlated negatively with age ($r = -0.69$, $P < 0.001$) (Table 1; Fig. 4).

By multiple regression analysis, of four potential independent determinants of blood acidity (P_bCO_2, NAE, age, and GFR), three were found to be statistically significant independent predictors of $[H^+]_b$, that is to say, P_bCO_2, NAE, and either age (multiple $R^2 = 0.46$) or GFR (multiple $R^2 = 0.44$), but not both age and GFR (Table 2). That is, when the regression model comprised the trio P_bCO_2, NAE, and age or the trio P_bCO_2, NAE, and GFR, the partial regression coefficients of all three predictors were significantly different from zero; but when both age and GFR were in the model, the partial regression coefficients of P_bCO_2, NAE, and age were significant but that of GFR was not (Table 2). Because age and GFR were highly correlated (Table 1; Fig. 4), it is not unexpected that together both variables would not be significant independent predictors.

Analyzing the relative importance of the separate independent variables in accounting for the overall variability in $[H^+]_b$, P_bCO_2 alone accounted for 19% of the variability ($R^2 = 0.19$) (Table 2). With NAE added, 26% of the variability in $[H^+]_b$ ($R^2 = 0.26$) was accounted for. With age added to P_bCO_2 and NAE, 46% of the variability in $[H^+]_b$ ($R^2 = 0.46$) could be accounted for. Once the effects of P_bCO_2 and NAE were considered, age and GFR were approximately equal in reducing unexplained variability in $[H^+]_b$ (R^2, 0.46 vs. 0.44), but because the two were highly correlated (Table 1; Fig. 4), together they offered little more than either alone ($R^2 = 0.48$) (Table 2).

The relative quantitative impact of the separate independent variables on $[H^+]_b$ was also assessed by comparing the standardized regression coefficients of the independent variables in the multiple regression

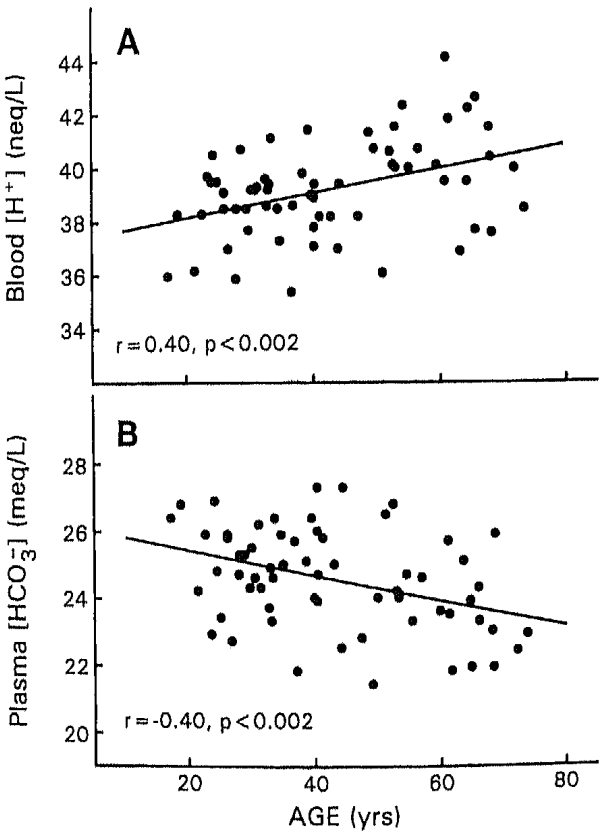

Fig. 2. Relation between $[H^+]_b$ and age (*A*) and between $[HCO_3^-]_p$ and age (*B*), in normal adult humans ($n = 64$). Each data point represents the mean steady-state value in a subject eating a constant diet. Regression equations: $[H^+]_b = 0.045 \cdot age + 37.2$; $[HCO_3^-]_p = -0.038 \cdot age + 26.0$.

[2] It might be argued that correlations among the three variables, $[H^+]_b$, P_bCO_2, and $[HCO_3^-]_p$, are inevitable because of the physicochemical dictates of the law of mass action as described by the Henderson-Hasselbalch equation. Clearly, within subjects, at equilibrium, the Henderson-Hasselbalch equation will be satisfied, and the value of any one of the three variables is predictable from those of the other two. But that does not mean that correlations between any two of the three variables are inevitable or even that the direction of the relationship when a correlation is present (sign of the correlation coefficient) is predictable. For example, with an appropriate intravenous infusion of $NaHCO_3$ (primary metabolic alkalosis) and a concomitant imposed restriction of pulmonary CO_2 excretion (primary respiratory acidosis), it is possible to produce a new equilibrium state of higher P_bCO_2 and $[HCO_3^-]_p$ without a change in $[H^+]_b$. With differing degrees of such isohydric hypercarbic-hyperbicarbonatemic combinations (within-subjects or among-subjects), at equilibrium the Henderson-Hasselbalch relation will always be satisfied, but there will be no correlation between the invariant $[H^+]_b$ and the differing values of either P_bCO_2 or $[HCO_3^-]_p$. More germane to the present study, the finding that both $[H^+]_b$ and $[HCO_3^-]_p$ correlated positively with P_bCO_2 among subjects (Tables 1 and 2) is not a predictable consequence of the Henderson-Hasselbalch relation. A P_bCO_2 increase can occur due to bicarbonate administration (respiratory suppression induced by the alkalemia attending bicarbonate administration), in which case $[HCO_3^-]_p$ would be observed to increase as $[H^+]_b$ decreases (negative correlation). Alternatively, a P_bCO_2 increase can occur because of primary respiratory insufficiency, in which case $[H^+]_b$ and $[HCO_3^-]_p$ both would be observed to increase (positive correlation). Thus the finding that in the steady state both $[H^+]_b$ and $[HCO_3^-]_p$ correlated positively with P_bCO_2 among subjects (Tables 1 and 2) indicates that differences in $[H^+]_b$ and $[HCO_3^-]_p$ among subjects reflect primary differences in the set-point at which P_bCO_2 is regulated among those subjects (20, 25).

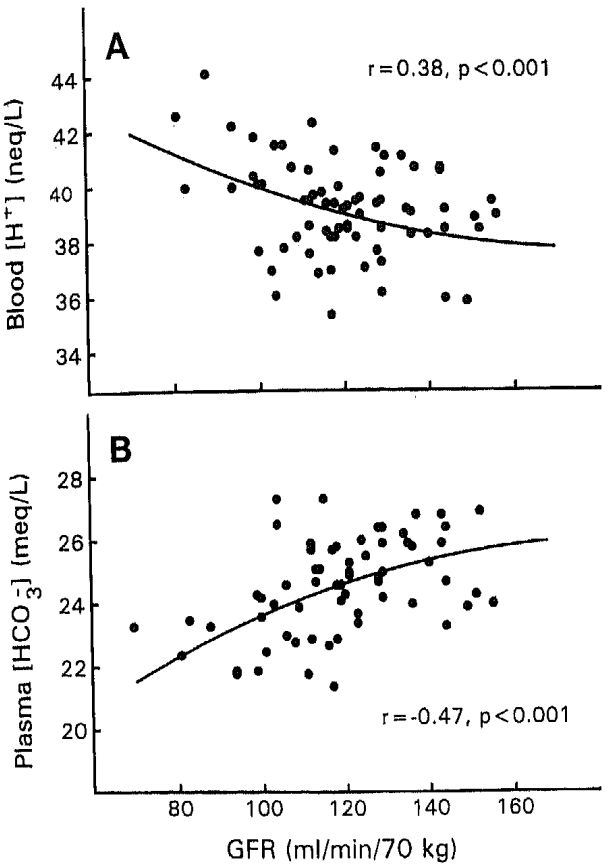

Fig. 3. Relation between $[H^+]_b$ and GFR (*A*) and between $[HCO_3^-]_p$ and GFR (*B*), in normal adult humans (n = 64). Each data point represents the mean steady-state value in a subject eating a constant normal diet. Although there was a statistically significant linear relation between $[H^+]_b$ and GFR ($[H^+]_b = -0.033 \cdot GFR + 43.0$; $r^2 = 0.11$), the variability accounted for was slightly greater with a nonlinear fit, that is, $[H^+]_b = 35.0 \cdot e^{12.9/GFR}$ ($r^2 = 0.14$). Similarly, the relation between $[HCO_3^-]_p$ and GFR was improved slightly by a nonlinear fit, that is, $[HCO_3^-]_p = 29.6 \cdot e^{-22.1/GFR}$ ($r^2 = 0.22$) vs. $[HCO_3^-]_p = 0.038 \cdot GFR + 19.9$ ($r^2 = 0.21$).

models (Table 2). With age, NAE, and P_bCO_2 in the model, P_bCO_2 had the greatest impact, age had nearly as great an impact, and NAE had the least impact (Table 2). With GFR, NAE, and P_bCO_2 in the model, P_bCO_2 again had the greatest impact, GFR had a lesser impact and in the opposite direction from P_bCO_2, and NAE again had the least impact (Table 2).

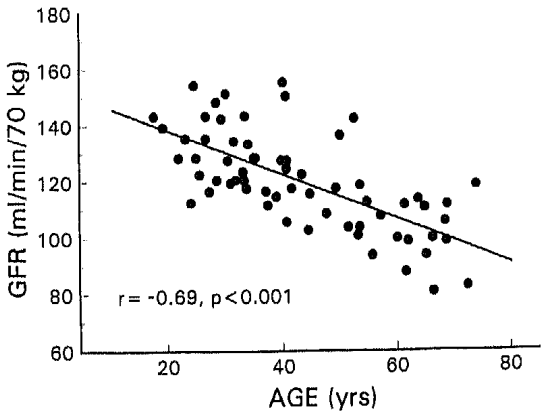

Fig. 4. Relation between GFR and age in normal adult humans (n = 64). Each data point represents the mean steady-state value in a subject eating a constant normal diet. Regression equation: GFR = $-0.79 \cdot age + 154$.

Table 2. *Summary of multiple regression analyses*

	P_bCO_2	NAE	GFR	Age	b_0	R^2	P
$[H^+]_b$							
b_i	+0.30				27.4	0.19	<0.001
B_i	+0.44						
P	<0.001						
$[H^+]_b$							
b_i	+0.33	+0.012			25.4	0.26	<0.001
B_i	+0.47	+0.27					
P	<0.001	0.020					
$[H^+]_b$							
b_i	+0.39	+0.012	−0.043		28.3	0.44	<0.001
B_i	+0.56	+0.26	−0.43				
P	<0.001	<0.01	<0.001				
$[H^+]_b$							
b_i	+0.39	+0.012		+0.051	22.1	0.46	<0.001
B_i	+0.56	+0.26		+0.45			
P	<0.001	<0.01		<0.001			
$[H^+]_b$							
b_i	+0.38	+0.013	−0.022	+0.034	24.7	0.48	<0.001
B_i	+0.54	+0.28	−0.22	+0.30			
P	<0.001	<0.005	0.10	<0.03			
$[HCO_3^-]_p$							
b_i	+0.42				8.4	0.50	<0.001
B_i	+0.76						
P	<0.001						
$[HCO_3^-]_p$							
b_i	+0.40	−0.008			9.9	0.55	<0.001
B_i	+0.675	−0.225					
P	<0.001	0.012					
$[HCO_3^-]_p$							
b_i	+0.36	−0.008	+0.027		8.1	0.65	<0.001
B_i	+0.61	−0.22	+0.325				
P	<0.001	<0.01	<0.001				
$[HCO_3^-]_p$							
b_i	+0.38	−0.009		−0.035	12.2	0.68	<0.001
B_i	+0.65	−0.28		−0.36			
P	<0.001	<0.002		<0.001			
$[HCO_3^-]_p$							
b_i	+0.37	−0.009	+0.012	−0.025	10.8	0.69	<0.001
B_i	+0.625	−0.24	+0.14	−0.26			
P	<0.001	0.002	0.179	0.012			

For descriptions of abbreviations, see legend to Table 1. *B*, standardized coefficients; *b*, nonstandardized coefficients. $Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + \ldots$; $Y = B_1 \cdot X_1 + B_2 \cdot X_2 + \ldots$.

The magnitude of the effect of age on $[H^+]_b$ over the range of ages studied was ~3 neq/l, which is an 8% increase in $[H^+]_b$ from age 17 to 74 yr (Table 3). This magnitude of effect of age difference on $[H^+]_b$ exceeded (by ~60%) that associated with the normal extremes of EAP and was less (by ~20%) than that associated with the normal extremes of P_bCO_2 (Table 3).

Plasma $[HCO_3^-]$. $[HCO_3^-]_p$ correlated negatively with age ($r = -0.39$, $P < 0.002$) and NAE ($r = -0.32$, $P < 0.01$) and positively with P_bCO_2 ($r = +0.71$, $P < 0.001$) and GFR ($r = +0.46$, $P < 0.001$) (Table 1; Fig. 2 and 3). As was the case for $[H^+]_b$, independent predictors of $[HCO_3^-]_p$ were P_bCO_2, NAE, and either age (multiple $R^2 = 0.68$) or GFR (multiple $R^2 = 0.65$) (Table 2), but not both age and GFR.

Analyzing the relative importance of the separate independent variables in accounting for the overall variability in $[HCO_3^-]_p$, P_bCO_2 alone accounted for 50% of the variability in $[HCO_3^-]_p$ ($R^2 = 0.50$) (Table 2). With NAE added to P_bCO_2, 55% of the variability in $[HCO_3^-]_p$

Table 3. *Impact analysis*

Predictors Fixed	Predictor Varied	Predictor Range		Dependent Variable	Predicted Value of Dependent Variable			
		Min	Max		Min	Max	Diff	%Diff
NAE, P_{CO_2}	Age	17.3	73.7	H^+	37.8	40.7	2.9	8
Age, P_{CO_2}	NAE	15.5	150.3	H^+	38.2	39.9	1.8	5
Age, NAE	P_{CO_2}	33.2	44.1	H^+	37.2	41.1	3.9	10
NAE, P_{CO_2}	GFR	81.0	156.0	H^+	40.8	37.6	−3.2	8
NAE, P_{CO_2}	Age	17.3	73.7	HCO_3^-	25.5	23.5	−2.0	−8
Age, P_{CO_2}	NAE	15.5	150.3	HCO_3^-	25.2	24.0	−1.2	−5
Age, NAE	P_{CO_2}	33.2	44.1	HCO_3^-	22.6	26.7	4.2	18
NAE, P_{CO_2}	GFR	81.0	156.0	HCO_3^-	23.5	25.6	2.1	9

Units: Age, yr; NAE, meq/day; P_{CO_2}, mmHg; GFR, ml·min^{-1}·70 kg body wt^{-1}; H^+, neq/l; HCO_3^-, meq/l. Min, minimum; Max, maximum; Diff, difference; %Diff, percent difference.

($R^2 = 0.55$) was accounted for. With age added to P_bCO_2 and NAE, 68% of the variability in $[HCO_3^-]_p$ ($R^2 = 0.68$) could be accounted for. Once the effects of P_bCO_2 and NAE were considered, age and GFR were approximately equal in reducing unexplained variability in $[HCO_3^-]_p$ (R^2, 0.68 vs. 0.65) (Table 2), but since the two were highly correlated (Fig. 4), together they offered little more than either alone ($R^2 = 0.69$).

The relative quantitative impact of the separate independent variables on $[HCO_3^-]_p$ was also assessed by comparing the standardized regression coefficients of the independent variables in the multiple regression models (Table 2). With age, NAE, and P_bCO_2 in the model, P_bCO_2 had the greatest impact, and age and NAE each had about one-half as much impact and in the opposite direction (Table 2). With GFR, NAE, and P_bCO_2 in the model, P_bCO_2 again had the greatest impact, GFR had about one-half as much impact and in the opposite direction from P_bCO_2, and NAE had the least impact (Table 2).

The magnitude of the effect of age on $[HCO_3^-]_p$ over the range of ages studied was approximately −2 meq/l, which is an 8% decrease from age 17 to 74 yr (Table 3). This magnitude of effect of age difference on $[HCO_3^-]_p$ exceeded (by ~60%) that of the normal extremes of EAP and was opposite in direction from that over the normal extremes of P_bCO_2 (Table 3).

Divergent effect of age and parallel effect of P_bCO_2 on $[H^+]_b$ and $[HCO_3^-]_p$. It may be difficult to envision how P_bCO_2 can correlate positively with both $[H^+]_b$ and $[HCO_3^-]_p$, given that $[H^+]_b$ correlates positively and $[HCO_3^-]_p$ correlates negatively with age. The difficulty lies in trying to envision simultaneously two three-dimensional relationships (one among $[H^+]_b$, P_bCO_2, and age; the other among $[HCO_3^-]_p$, P_bCO_2, and age), given only their several component two-dimensional projections. One way around the difficulty is to look at the two three-dimensional relationships directly, as depicted in Fig. 5.

Figure 5*A* shows $[H^+]_b$ as the dependent variable (y-axis), with age (x-axis) and P_bCO_2 (z-axis) as the two independent variables. Figure 5*B* is identical except that $[HCO_3^-]_p$ is the dependent variable (y-axis). In both graphs, the data points tend to be distributed in a plane, the "best-fit" plane, which is analogous to the "best-fit" line in a two-variable graph.

Note that in both graphs, the planes slope upward as P_bCO_2 increases from 34 to 46 mmHg, i.e., that both $[H^+]_b$ and $[HCO_3^-]_p$ correlate positively with P_bCO_2. Yet, in the case of $[H^+]_b$ (Fig. 5*A*), the plane slopes upward as age increases from 20 to 80 yr, i.e., $[H^+]_b$ correlates positively with age, whereas in the case of $[HCO_3^-]_p$ (Fig. 5*B*), the plane slopes downward as age increases over the same range, i.e., $[HCO_3^-]_p$ correlates negatively with age.

Serum chloride. Serum chloride concentration correlated positively with age ($r = +0.51$, $P < 0.001$) (Fig. 6) and negatively with GFR ($r = -0.33$, $P < 0.01$) and $[HCO_3^-]_p$ ($r = -0.30$, $P < 0.02$). Serum unmeasured anion concentration, calculated as $Na^+ - (Cl^- + HCO_3^-)$, did not vary with age, GFR, or $[HCO_3^-]_p$.

DISCUSSION

The results of the present study identify age as a determinant of the acid-base composition of the blood in adult humans. From young adulthood to old age, otherwise healthy men and women develop a progressive increase in blood acidity and decrease in $[HCO_3^-]_p$, indicative of an increasingly worsening low-grade metabolic acidosis.

The observed changes in $[H^+]_b$ and $[HCO_3^-]_p$ with age occur independently of the only other known determinant of the metabolic component of systemic acid-base equilibrium in normal adult humans, that is, dietary net acid load (i.e., EAP) (20). That is, the age-related increase in acidity and decrease in bicarbonate concentration were evident when dietary net acid load, as reflected in steady-state NAE, was held constant (Table 2). Because diet net acid load was always positive in the subjects studied, however, it remains to be determined whether an age-related change in acid-base status would occur among subjects ingesting non-net-acid-producing diets.

Although diet acid load is an independent determinant of blood acid-base composition, as we previously demonstrated (20) and confirm in this study (Tables 2 and 3), the acid-base effect of extremes of age (17–74 yr) is ~1.6 times greater than that of extremes of normal dietary acid loads (15–150 meq/day) (Table 3). Increasing age, therefore, greatly amplifies the chronic low-

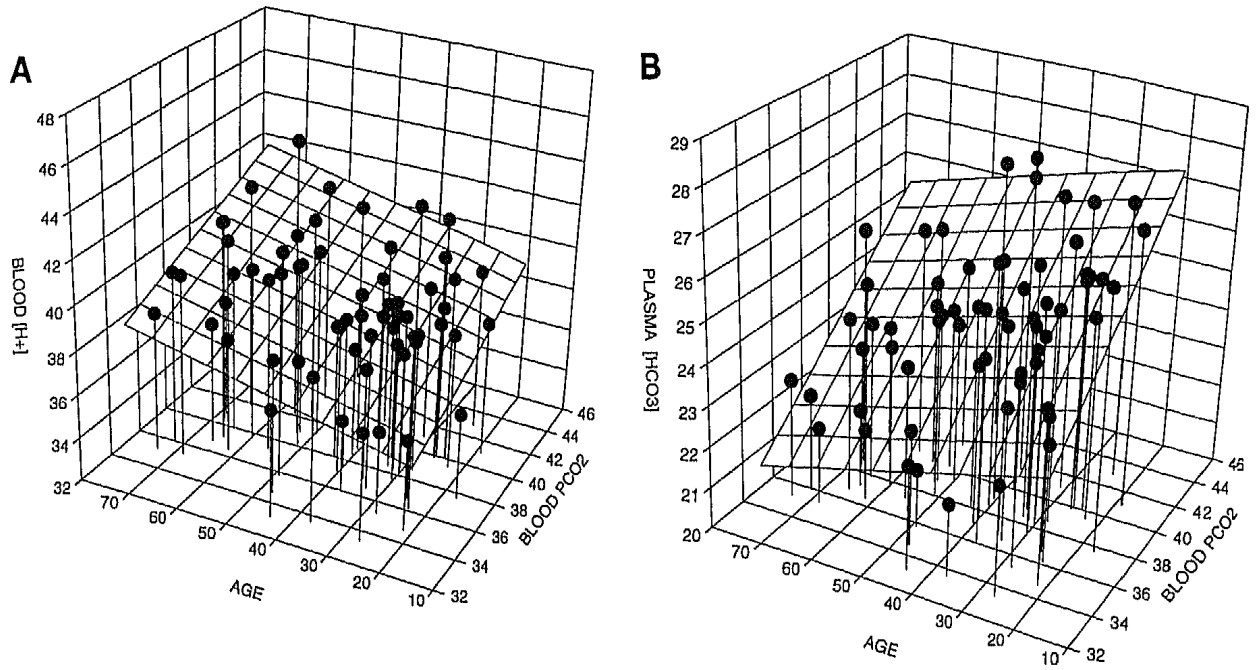

Fig. 5. Relationship between $[H^+]_b$ as the dependent variable (y-axis), with age (x-axis) and P_bCO_2 (z-axis) as the two independent variables (A) and between plasma $[HCO_3^-]_p$ as the dependent variable (y-axis) with age (x-axis) and P_bCO_2 (z-axis) as independent variables (B). In both A and B, the data points tend to be distributed in a plane, the "best-fit" plane, which is analogous to the best-fit line in a two-variable graph. In both graphs, the planes slopes upward as P_bCO_2 increases from 34 to 46 mmHg, i.e., that both $[H^+]_b$ and $[HCO_3^-]_p$ correlate positively with P_bCO_2. Yet, in the case of $[H^+]_b$, the plane slopes upward as age increases from 20 to 80 yr, i.e., $[H^+]_b$ correlates positively with age, whereas in the case of $[HCO_3^-]_p$ the plane slopes downward as age increases over the same range, i.e., $[HCO_3^-]_p$ correlates negatively with age.

grade metabolic acidosis induced by the diet acid load (20, 35) and presumably also amplifies its pathophysiological consequences (see below).

Cross-sectional studies by earlier investigators, who considered the question of age and blood acid-base composition, failed to detect a correlation. Yet, few laboratories reported data from subjects with an age range that spanned young adulthood to old age, and in most cases the number of subjects was too few for meaningful analysis (12). Shock and Yiengst (38), who studied 152 subjects spanning ages 40 to 89 yr, noted an age-related trend in blood pH but not in $[HCO_3^-]_p$. Our reanalysis of their data, however, suggests that $[HCO_3^-]_p$, too, declines with age (12).

The results of cross-sectional analyses must be interpreted cautiously. The finding that $[H^+]_b$ is higher and $[HCO_3^-]_p$ lower in older subjects does not permit one to conclude that individual subjects increase their $[H^+]_b$ and decrease their $[HCO_3^-]_p$ as they get older. Longitudinal studies are required for that.

Although there were no age-related differences in P_bCO_2 among subjects, there were substantial differences in P_bCO_2 among subjects at every age, and those differences affected blood acid-base status among subjects (Tables 1 and 2; Fig. 5). The finding that both $[H^+]_b$ and $[HCO_3^-]_p$ correlated positively with P_bCO_2, however, is not a new finding, having been well described by Madias et al. (24) and Kurtz et al. (20).[3] The

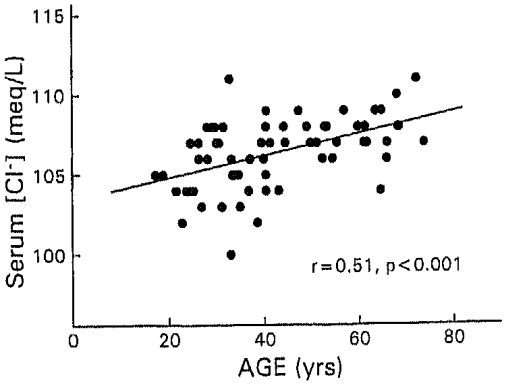

Fig. 6. Relation between serum chloride concentration ($[Cl^-]$) and age in normal adult humans (n = 64). Each data point represents the mean steady-state value in a subject eating a constant normal diet. Regression equation: serum $[Cl^-] = 0.075 \cdot age + 103$.

[3] One way to understand the finding is to arbitrarily consider as the "normal" P_bCO_2 the midpoint (40 mmHg) of the normal range (35–45 mmHg), and then consider that subjects who regulate at lower values (35–39 mmHg) have mild primary respiratory alkalosis (hyperventilation) and those who regulate at higher values (41–45 mmHg) have mild primary respiratory acidosis (hypoventilation). That is, some people tend to be mild hypoventilators and others mild hyperventilators, with all degrees of variation in between. The differences in ventilatory set point among subjects clearly do not reflect responses to primary differences in blood acidity, because then P_bCO_2 would correlate inversely with $[H^+]_b$ (more acidity, more ventilation, lower P_bCO_2), whereas the finding is that P_bCO_2 correlates directly with $[H^+]_b$ (20, 24). It is the P_bCO_2 differences that are primary. That is, *1*) higher P_bCO_2 values indicate more CO_2 retention, higher H_2CO_3 concentrations, and consequently higher $[H^+]_b$ and $[HCO_3^-]_p$; and *2*) lower P_bCO_2 values indicate more CO_2 excretion, lower H_2CO_3 concentrations, and consequently lower $[H^+]_b$ and $[HCO_3^-]_p$. Thus $[H^+]_b$ and $[HCO_3^-]_p$ both correlate directly with P_bCO_2.

finding that the relationship holds at all ages (see Fig. 5) extends their findings.

It is also evident from Fig. 5 (and Tables 1 and 2) that the age effect on $[H^+]_b$ and $[HCO_3^-]_p$ is independent of P_bCO_2. Both subjects who are mild chronic hypoventilators and those who are mild chronic hyperventilators develop an increasing degree of low-grade metabolic acidosis with age.

A progressively increasing degree of metabolic acidosis developing with advancing age might be regarded as predictable, inasmuch as renal functional integrity progressively deteriorates with age (8, 23). The kidney is a major determinant of the set-point at which $[HCO_3^-]_p$ is regulated (14, 22), and with advancing age, a condition like that of chronic renal insufficiency develops (8, 23). Renal insufficiency induces metabolic acidosis variably due to reduced conservation of filtered bicarbonate and reduced excretion of acid (7, 13, 14, 25, 34, 36). In older humans, acid-excretory ability in response to acute exogenous acid loading is impaired (1, 2, 28). With more prolonged acid loading, a more severe degree of metabolic acidosis is sustained compared with that in similarly acid-loaded younger subjects (18).

It is not surprising, therefore, to find that elderly persons, like patients with chronic renal insufficiency, regulate their plasma acidity at significantly higher levels and their $[HCO_3^-]_p$ at significantly lower levels, compared with the levels in younger persons (12). In the present study we tested whether the observed age-related changes in acid-base composition were related to the age-related decline in renal function. The increases in blood acidity and decreases in $[HCO_3^-]_p$ with age correlated significantly with the observed decline in GFR, independently of changes in EAP (Tables 1 and 2; Fig. 3).

Based on the relationship between degree of renal insufficiency and degree of metabolic acidosis reported in patients with mild chronic renal disease, the degree of age-related renal insufficiency in our subjects is sufficiently great to account for the observed age-related change in acid-base composition (17, 39). The finding that serum chloride concentration increased in relation to the observed declines in GFR and $[HCO_3^-]_p$, is consistent with a renal mechanism causing the worsening age-related metabolic acidosis. Hyperchloremia with little or no change in unmeasured anion concentration is characteristic of mild-to-moderate renal insufficiency regardless of etiology (17).

Holding both EAP and P_bCO_2 constant, both GFR and age satisfied the criteria of an independent determinant of $[H^+]_b$ and $[HCO_3^-]_p$ (Table 2). Since GFR and age were significantly correlated (Table 1; Fig. 4), however, when the two were considered together, either but not both satisfied the criteria of independent predictors (Table 2). GFR may only provide a rough estimate of age-related changes in renal acid-base regulatory function, which is primarily a function of the renal tubule. When both GFR and age were considered together as potentially independent predictors along with P_bCO_2 and EAP, the age effect remained significant with GFR held constant, but not vice versa (Table 2). Conceivably, nonrenal age-related factors [e.g., base release by bone (16)] also influence plasma acid-base composition.

The present results provide the first demonstration that, in normal subjects eating ordinary acid-producing diets, small reductions in GFR within the normal range can have a metabolic acidosis-producing effect. Just as the relatively small acid loads from ordinary diets induce discernible metabolic acidosis in subjects with normal GFR (20), the relatively small reductions of GFR accompanying aging induce discernible metabolic acidosis in subjects with normal dietary acid loads (Fig. 3). Thus both acid-loading metabolic acidosis and renal metabolic acidosis exist to a predictable degree as the norm in adult humans who eat ordinary acid-producing diets and whose renal function declines with age.

Although dietary acid load is a significant independent determinant of plasma acid-base composition (Table 2), age-related differences in dietary acid load did not explain the observed age-related changes in plasma acid-base composition (Table 1). The effect of differences in EAP on systemic acid-base equilibrium is independent of P_bCO_2 (Table 2), as we previously reported (20). The present studies now reveal that the effect occurs even when age and GFR are held constant (Table 2; Fig. 7).

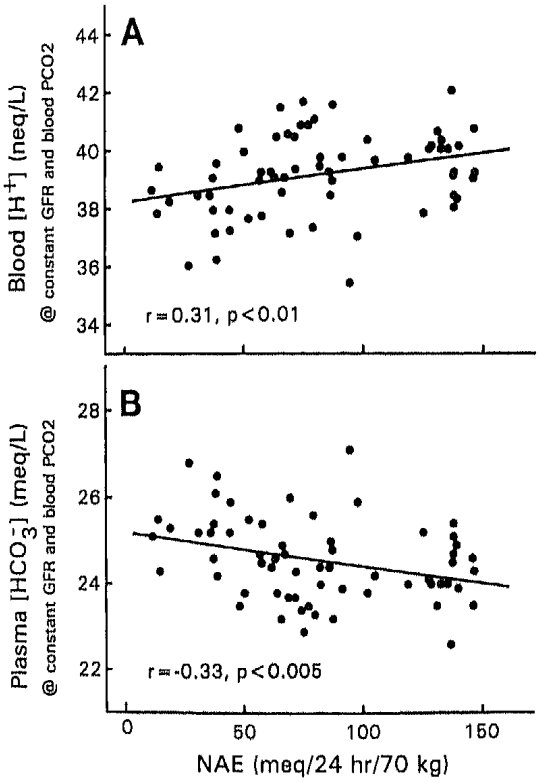

Fig. 7. Relation between $[H^+]_b$ and NAE (*A*) and between $[HCO_3^-]_p$ and NAE (*B*), in normal adult humans (n = 64). The correlation between $[H^+]_b$ and NAE was of only borderline significance when the effects of P_bCO_2 and GFR were not taken into consideration (Table 2), whereas when adjusted for P_bCO_2 and GFR, NAE was a highly significant determinant of $[H^+]_b$. The plot depicts the values of $[H^+]_b$ when P_bCO_2 and GFR were held constant (at their respective mean values for the group: P_bCO_2 = 38.5 mmHg, GFR = 120 ml·min^{-1}·70 kg^{-1}). $[HCO_3^-]_p$ was similarly treated. With these adjustments, the regression equations are as follows: $[H^+]_b$ = −0.042·GFR + 44.2; $[HCO_3^-]_b$ = +0.027·GFR + 21.7.

Additional acidosis-producing factors which we did not evaluate are a wide variety of normally occurring age-related changes in circulating levels of hormones and body composition that potentially directly and/or indirectly influence blood acid-base composition.

One might question whether the magnitude of the observed age-related changes in $[H^+]_b$ and $[HCO_3^-]_p$ are sufficiently large to have pathophysiological significance. Yet, the acid-base effect of age is sufficiently great to result in such pathophysiological sequelae of metabolic acidosis as accelerated rates of bone resorption and reduced rates of bone formation (35), nitrogen wasting (10), and suppression of growth hormone secretion (11), all of which are reversible in part by returning plasma acid-base composition to its more youthful state (10, 11, 35). Furthermore, the age-related changes in blood acid-base composition are greater than those occurring in many patients with classic renal tubular acidosis, in particular those with the syndrome of incomplete distal renal tubular acidosis, in whom disturbances of blood acid-base composition are barely discernible yet in whom alkali-reversible sequelae of metabolic acidosis characteristically occur (31, 32).

As Alpern (3) has noted, the degree of metabolic acidosis as manifested by the magnitude of change in plasma acid-base composition underestimates the severity of the pathophysiological injury caused by the acid-base disturbance. With diet-dependent chronic metabolic acidosis, renal and extrarenal homeostatic adaptations occur that serve to minimize disturbances in $[H^+]_b$ and $[HCO_3^-]_p$ but in which the adaptations themselves have detrimental effects. These include decreased renal citrate production and excretion (15), hypercalciuria (21), dissolution of bone (4–6, 9, 19), protein catabolism and muscle wasting (26, 30), and progression of renal disease (29). Thus homeostatic mitigation of the disturbance of extracellular acid-base equilibrium requires the body to accept certain deleterious "trade-offs" (3). Although the degree of age-related, diet-dependent metabolic acidosis is mild as judged by the degree of perturbation of blood acid-base equilibrium, it cannot be considered mild as judged by its negative biological effect.

Therefore the finding that we are all becoming more and more acidotic as we age would seem to be of real concern. Yet, at the same time, the finding offers a novel and testable therapeutic approach to age-related disorders of bone, muscle, and kidney, namely, sustained correction of acidosis by chronic alkali administration and/or appropriate dietary modification.

The novel findings of the present study can be summarized as follows: *1*) as they age, normal adult humans develop a progressively worsening low-grade chronic metabolic acidosis; *2*) age has a greater metabolic acidosis-producing effect than does diet acid load, the only other factor known to affect the metabolic component of acid-base equilibrium in normal adult humans; *3*) age-related metabolic acidosis is not accounted for by differences in diet acid load among subjects; *4*) the occurrence of age-related metabolic acidosis is independent of the set-point at which the respiratory system regulates P_{CO_2} independently of blood acidity, i.e., it occurs both in subjects who tend to be mild hypoventilators and those who tend to be mild hyperventilators; *5*) the severity of age-related metabolic acidosis parallels and is largely accounted for by the normally occurring age-related decline in renal function in adult humans; *6*) the severity of age-related metabolic acidosis is within the range that would be predicted for the degree of age-related renal insufficiency, based on the known relationship between acidosis-severity and degree of renal insufficiency in patients with renal disease; and *7*) the reduction in $[HCO_3^-]_p$ that occurs with age-related metabolic acidosis is accompanied by a reciprocal increase in plasma chloride concentration (hyperchloremia), consistent with the metabolic acidosis of patients with early renal disease or renal tubular acidosis.

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