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The concentration of invidual monocarboxylic short chain fatty acids in serum of normal children

By

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The normal serum concentrations of short-chain fatty acids C_2—C_6 (acetic to n-caproic) were estimated by gas chromatography in 35 children aged 4—16 years. Individual fluctuations were wide. The highest mean level concerned acetic acid (6.98 + 3.71 μg/ml), whereas the mean concentrations of other SFA were considerably lower and did not exceed 1.0 μg/ml except for n-caproic acid (1.34 μg/ml).

Since the discovery of metabolic disorders leading to the accumulation of some short chain fatty acids (SFA) in biological fluids [1, 6, 14, 15] much attention has been paid to the evaluation of these compounds. The total fatty acids in human serum consist of a number of individual free fatty acids (FFA) with various chain length, mainly C_{12}—C_{22} [4]. SFA with 2 to 6 carbon atoms represent a small fraction of FFA; they nevertheless play an important role being intermediates in some biochemical pathways. On the other hand an excess of SFA in the organism exerts a toxic effect on cerebral and nervous tissues by inhibiting their metabolic activity [13, 14, 15]. The metabolism of SFA was found to be abnormal in cases of liver failure and cyclic vomiting [2, 10].

Among the techniques available for the identification, separation and quantitation of SFA, the gas-liquid chromatographic procedure seems the most convenient one [3, 5, 7, 8, 9, 12]. The purpose of this investigation was to determine the normal values of individual SFA C_2—C_6 in serum of children.

MATERIAL AND METHODS

The sera of 35 children (21 boys and 14 girls) ranging in age from 4 to 16 years, hospitalized for orthopaedic diseases were studied. The patients were free from metabolic disorders, infections, disturbances of development, malnutrition, renal and mental disorders. They received no drugs at the time of the study.

Blood was obtained by venipuncture after fasting overnight. Two half samples of serum, separated by centrifugation, were placed in 50 ml flasks and acidified

to pH 3 by the dropwise addition of 1 M ortophosphoric acid. Exactly 25 μg of n-heptanoic acid (1 ml of dilute stock solution of n-heptanoic acid in water) was added as an internal standard. The sample was then immediately steam-distilled in Kjeldahl's semimicro apparatus until 30 ml of distillate had collected in a graduated cylinder kept in ice. In order to bind the traces of fatty acids eventually present in the water, some NaOH was added. The distillation procedure required 7—10 min. The distillates were promptly adjusted to pH 7.5 by 0.1 M NaOH in order to convert the volatile fatty acids to their non-volatile sodium salts. The neutralized distillates were then placed in 150 ml flasks, evaporated and the residue was dissolved in 1 ml of distilled water, transferred to conical tubes and dried in a vacuum desiccator over KOH at 85 °C for 5—6 hours, then stoppered and stored at —20 °C until analysis. Prior to chromatography the sodium salts were re-converted to free acids by adding 50 μl of 25% H_3PO_4 to the dry sample. After 5 min. the solution was thoroughly stirred and 10 μl of it was injected into the gas-chromatograph with Hamilton's microsyringe.

A gas chromatograph G.C.H.F. 18.3. (Chromotron) equipped with flame ionization detector was used. A single 3 m × 4 m steel column packed with 5% SP 1000 (Supelco Inc.) on Chromosorb GAW DMCS 80/100 mesh was employed and maintained under isothermic conditions. The temperatures were, column —160 °C; injector and detector —200 °C. The carrier gas was N_2, 70 ml/min. The flow rates for H_2 and air were 25 ml/min and 750 ml/min respectively. The analyses were carried out at sensitivities 1×10^{-10} AFS to 30×10^{-10} AFS according to the composition of the sample.

Identification of SFA and calculation of chromatograms

Identification of separated SFA was based on the chromatograms of an artificial standard mixture containing acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, iso-caproic, n-caproic and n-heptanoic acids (Fluka, Buchs, Switzerland) in water, at a concentration of 10 μg/ml. The peak areas were measured by planimetry. The factors for the relative peak areas of each SFA (mass correction factors) were calculated by chromatographing known amounts of the investigated SFA simultaneously with 25 μg of n-heptanoic acid as an internal standard. Quantitation of the amount of each SFA was based on the formula,

$$m_x = \frac{A_x \cdot f_x \cdot m_w}{A_w \cdot V} \ (\mu g/ml), \text{ where}$$

m_x — concentration of SFA in μg/ml
A_x — peak area of SFA
f_x — mass correction factor
m_w — amount of internal standard in the sample (25 μg)
A_w — peak area of the internal standard
V — volume of serum taken for analysis

TABLE I

Mass correction factors of SFA for the flame-ionization detector as related to n-heptanoic acid

Acetic acid	0.71
Propionic acid	0.79
Iso-butyric acid	1.02
n-butyric acid	1.05
Iso-valeric acid	1.07
n-valeric acid	0.98
Iso-caproic acid	1.08
n-caproic acid	0.99
n-heptanoic acid	1.00

RESULTS

Table II presents the results in comparison to data reported by some other authors. Acetic acid was present in every sample and showed the highest

TABLE II

Normal serum concentrations of individual short-chain fatty acids (μg/ml)

Authors	Subjects		Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Iso-caproic	Caproic
Perry et al (11).	31 adults	Range	1.85—8.6	tr—1.8	0—0.22	0—0.29	0—0.42			0—0.32
		Mean	4.65	0.32						
		S. D.	1.66	0.37						
Tanaka et al (14).	4 children	Range								
		Mean		0.28	0.27	0.12	0.58	0.04		0.16
		S. D.		0.07	0.10	0.11	0.22	0.03		0.10
	4 adults	Range								
		Mean		0.25	0.28	0.10	0.94	0.22		0.44
		S. D.		0.03	0.02	0.0	0.29	0.03		0.07
Papierkowski et al.	35 children 4—16 year	Range	2.12—14.7	0—1.1	0—1.14	0—1.65	0—2.52	0—1.78	0—1.97	0—4.26
		Mean	6.98	0.50	0.40	0.73	0.79	0.69	0.27	1.34
		S. D.	3.71	0.31	0.28	0.40	0.62	0.48	0.51	0.72
		V. C.	53.1	62.0	70.0	54.7	78.4	69.5	188.8	53.7

S. D. — standard deviation

V. C. — variation coefficient in per cent

ts — trace

mean level, amounting to 6.98 ± 3.71 μg/ml. The concentrations of other SFA were considerably lower, not exceeding the mean value of 1.0 μg/ml, except for n-caproic acid which amounted to 1.34 ± 0.72 μg/ml. N-butyric, iso-valeric and n-caproic acids were detected in the sera of 34 subjects, iso-butyric acid in 33, propionic and n-valeric acids in 32, and iso-caproic acid in 18 sera. The concentration of all SFA studied showed considerable individual fluctuations. Acetic and n-caproic acids showed little variability (V. C. 53.1 and 53.7% respectively), while iso-caproic acid was the most variable (V. C. = 188.8%).

Discussion

Information is scarce and diverging concerning the normal concentration of individual SFA in human serum, due presumably to differences in techniques [1, 12, 15]. Tanaka et al. [15] studying patients with isovaleric acidaemia compared the results with the serum SFA levels observed in 8 control subjects (4 children and 4 adults). With the exception of isovaleric acid in adults, the concentrations of individual SFA were lower than those found in our material. The normal values for SFA found by Perry et al. [12] in adults were also much lower. According to Kuntz et al. [7], acetic, propionic, iso-butyric and iso-valeric acids are always, whereas n-butyric, n-valeric and n-caproic acids are occasionally, detectable in normal human serum. Perry et al. [12] showed that acetic acid was present in all sera yielding the largest peak on gas chromatograms. The same was noted in our children.

To our best knowledge no investigations have been carried out to evaluate the behaviour of iso-caproic acid in serum. In view of our results it may be a normal but very labile constituent. N-valeric acid, the compound used by Perry et al. [12] as an internal standard, is mostly present in normal serum [1, 7, 15], and we too have found it in 33 of the 35 children. We have therefore employed n-heptanoic acid as an internal standard as this acid does not occur in biological fluids.

All the SFA values showed a wide scatter and high standard deviations and variation coefficients. The same was the case with individual middle and long-chain fatty acids and total FFA [4]. This would point to the lability of the serum SFA levels, owing to differences in dietary habits and to the different metabolic rates of fatty acid synthesis and breakdown. It is also possible that differences exist in the rate of fatty acid release from adipose tissue and of their disappearance from the blood stream [4].

The normal values for monocarboxylic SFA can be useful in further investigations of some pathological states connected with deviations of lipid metabolism as well as in studies of metabolic acidosis caused by the accumulation of SFA in biological fluids.

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