

Determination of retinoic acid and retinol at physiological concentration by HPLC in Caucasians and Japanese women

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A sensitive, specific and simple method for simultaneous determination of retinoic acid and retinol in human serum by reverse phase HPLC with UV detector has been developed. Retinoic acid is measurable at physiological concentration in human serum by this method. There is no significant difference in serum retinoic acid level between Caucasian and Japanese subjects. However, the serum retinol level in Japanese women is significantly lower than that in Caucasian women

Key words: Retinoic acid, retinol, HPLC, Japanese, Caucasian

Introduction

Retinoic acid and retinol status are related to immune function, differentiation of epithelial tissues, cancer and mortality among children¹⁻⁵. The public health and clinical measurement of fat soluble vitamins are assuming more importance⁶. In the present study we sought:

- to develop a simple HPLC method for the simultaneous assessment of retinoic acid and retinol status, applicable to clinical and population based studies; and
- to assess the status of these compounds in those with disparate food cultures

Subjects and methods

The study involved 28 apparently healthy Caucasians (14 men and 14 women from Melbourne, Australia) and 21 age matched healthy Japanese women from Nagoya, Japan (Table 1).

The reproducibility and recovery of retinoic acid and retinol are shown in Table 2. The coefficients of variation (CV) of retinoic acid and retinol for 6 replicate analysis of serum were 8.6% and 4.4% respectively. Known amounts of retinoic acid and retinol were added to real samples (serum) and recovery rate of retinoic acid was $98.2 \pm 1.7\%$, with a range from 94-105%, and a CV of 4.1%. The recovery rate of retinol was $100.2 \pm 1.9\%$ with a range from 96-108% and CV of 4.5% (Table 2).

Table 2. Reproducibility and recovery of retinoic acid and retinol (Mean \pm SEM).

	Reproducibility	Recovery
	N=6 CV%	N=6 %
Retinoic Acid	8.6	$98.2 \pm 1.7\%$
Retinol	4.4	$100.2 \pm 1.9\%$

Serum retinoic acid was not significantly different between Caucasian men and women, nor between Caucasian women and Japanese women. However, serum retinol was relatively lower in Japanese women than Caucasian women (Table 3).

Table 3. Retinoic acid and retinol levels in human serum (Mean \pm SEM).

Subjects	N	Sex	Retinoic Acid $\mu\text{mol/L}$	Retinol $\mu\text{mol/L}$
Caucasian	14	M	0.034 ± 0.004	2.39 ± 0.16
	14	F	0.038 ± 0.005	$2.57 \pm 0.18^{**}$
Japanese	21	F	0.031 ± 0.004	1.84 ± 0.08

N is the number of subjects; the mean \pm SEM are shown; the significant difference between Caucasian and Japanese women is indicated by $**p < 0.001$.

Table 1. The age, stature, body weight and body mass index (BMI) of Caucasian and Japanese. (Mean \pm SEM)

Subjects	N	Sex	Age	Stature (cm)	Weight (kg)	BMI
Caucasian	14	M	49 ± 3 (37-64)	176 ± 2 (169-182)	74 ± 2 (56-89)	24.0 ± 0.7 (17.8-28.9)
	14	F	49 ± 2 (40-62)	163 ± 1 (154-170)	63 ± 3 (39-94)	23.8 ± 1.4 (14.5-35.3)
Japanese	21	F	50 ± 1 (41-59)	155 ± 1 (143-161)	54 ± 1 (43-64)	22.5 ± 0.6 (16.8-28.1)

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Discussion

A sensitive, specific and simple method for simultaneous evaluation of retinoic acid and retinol in human serum by reverse phase HPLC with UV detection has been developed.

Retinoic acid is measurable in physiological concentration in human serum by this method, at a molar concentration about 1.5% of that of retinol.

It is known that food intake and homeostatic mechanisms influence serum retinol and both may account for the differences between Caucasians and Japanese women in this study⁷. However, despite the different food intake patterns of these two groups, retinoic acid concentrations are the same, which suggests that homeostatic control of serum retinoic acid is important. It is also clear that retinoic acid status does not necessarily parallel that of retinol.

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用高壓液層析法測定高加索（白人）婦女與日本婦女 血清視黃酸和視黃醇的生理濃度

摘要

本文介紹了一個同時測定人體血清視黃酸（Retinoic acid）和視黃醇（Retinol）的簡單、特異和敏銳的方法。這個高壓液層析法是用紫外檢測器進行的，它可以測出血清中視黃酸的生理濃度。在高加索（白人）婦女與日本婦女之間沒有發現視黃酸有明顯的差異，但是，日本婦女血清中視黃醇卻明顯低於高加索婦女（ $p < 0.001$ ）。

要約

螢光検出付逆相高速液体クロマトグラフィーを用いてヒト血清のレチノイン酸とレチノールを高感度で特異的かつ簡便に測定できる方法を開発した。レチノイン酸は生理的濃度で測定可能で、レチノールの約1.5%であった。本法で在豪コーカサス男女、および日本在住日本女性の血清の両ビタミン濃度を測定した結果、血清レチノイン酸濃度は3グループ間に有為差がなかったが、レチノール濃度は日本女性がコーカサス女性よりも有為に低かった（ $p < 0.01$ ）。血清レチノイン酸には生体恒常性があるものと考察された。

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