

Correlation of serum L-carnitine and dehydro-epiandrosterone sulphate levels with age and sex in healthy adults

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Abstract

Objectives: L-carnitine and dehydro-epiandrosterone (DHEA) independently promote mitochondrial energy metabolism. We therefore wondered if an age-related deficiency of L-carnitine or DHEA may account for the declining energy metabolism associated with age.

Methods: we evaluated serum levels of L-carnitine and the sulphated derivative of DHEA (DHEAS) in cross-sectional study of 216 healthy adults, aged 20–95.

Results: serum DHEAS levels declined, while total carnitine levels increased with age ($P < 0.0001$). Total and free carnitine and DHEAS levels were lower in women than men ($P < 0.0001$). Esterified/free (E/F) carnitine (inversely related to carnitine availability) increased with age in both sexes ($P = 0.012$).

Conclusion: reduced carnitine availability correlates with the age-related decline of DHEAS levels. These results are consistent with the hypothesis that decreased energy metabolism with age relates to DHEAS levels and carnitine availability.

Keywords: carnitine, dehydro-epiandrosterone sulphate, gerontology, mitochondrial energy metabolism, sex

Introduction

Ageing is associated with a decline in activity of enzymes required for energy metabolism. For example, enzymes required for the tricarboxylic acid cycle and fatty acid oxidation decline with age [1]. The cause of this decline is unknown; however, an age-associated diminution of compounds that induce these mitochondrial enzymes would result in a parallel decrease of enzyme activity. Two such potential compounds are L-carnitine and dehydro-epiandrosterone (DHEA). These compounds promote mitochondrial and cellular energy production, principally via their effect on fatty acid metabolism. Thus, as L-carnitine availability and DHEA level decline with age, presumably mitochondrial energy production will also decrease.

L-Carnitine is an essential cofactor for the transport

of long-chain fatty acids across the inner mitochondrial membrane into mitochondrial matrix, making them available for β -oxidation, which is the most efficient metabolic path for energy production [2]. Thus L-carnitine facilitates energy availability, and is especially important for those tissues with high energy requirements. Cardiac and skeletal muscles are two such tissues and are the major storage sites of carnitine, which is primarily synthesized by the liver. Carnitine decreases in muscle with age [3–5] and this reduction might be related to the age-associated decline in mitochondrial function. Carnitine deficiency affects these tissues, whether the energy is used for mechanical (e.g. muscle) or anabolic (e.g. liver) work. Carnitine deficiency can therefore lead to cardiomyopathy, hepatic encephalopathy and skeletal muscle weakness [6]. Serum carnitine deficiency has been defined as an

esterified/free (E/F) carnitine ratio of either greater than 0.33 [7] or 0.4 [8]. A change in the serum E/F carnitine ratio may be an early marker for impending carnitine deficiency in tissue and thus predict the development of muscle or hepatic pathophysiology [9]. Even though the E/F carnitine ratio may provide clinically useful information, few data exist describing serum carnitine levels by age or sex in people [10].

DHEA is a steroid hormone that is primarily produced in the adrenal cortex as an intermediate in the biosynthesis of the sex hormones. It is the most abundant circulating steroid hormone, and exists almost exclusively as its sulphated derivative (DHEAS) [11]. Conditions such as obesity, hypercholesterolaemia and atherosclerosis have been associated with the age-associated decline of DHEA and DHEAS levels [12–15]. *In vivo* and *in vitro* administration of DHEA and DHEAS increases synthesis and activity of enzymes that mediate fatty acid oxidation and energy production, such as carnitine acyltransferases, which require carnitine as substrate. [16, 17]. Moreover, since DHEA and DHEAS modulate peroxisome activity [17], they could promote β -oxidation of very-long-chain fatty acids, making them available for mitochondrial β -oxidation when appropriately shortened. Taken together, these data suggest that DHEAS plays a role in energy metabolism through both a carnitine-dependent and a carnitine-independent mechanism. However, there are no reports studying the relationship of DHEAS and carnitine in the context of age or sex.

Because DHEAS and carnitine are important for energy metabolism, which declines with age, we hypothesized that serum carnitine and DHEAS covaried as a function dependent on age. We therefore examined serum carnitine and DHEAS levels across the healthy adult age span in men and women.

Methods

Subjects

Subjects were recruited for an unrelated vaccine study from the Madison, WI, area. Inclusion criteria included (i) being ambulatory, (ii) living independently in the community and (iii) being free of acute disease (based on history and physical examination). Subjects were divided into two groups: young (20–60 years old) and old (>60 years old). Young subjects were free of chronic disease and taking no prescription medication other than birth control pills. Older subjects took no prescription medication known to effect the immune system and were free of chronic diseases such as diabetes mellitus and arterial disease (based on verbal medical history) that required prescription medication other than single agents for hypertension. Brief physical examination of each subject revealed no

important findings. The study was approved by the institutional review board and informed written consent was given.

Carnitine

Serum carnitine was measured in triplicate by a quantitative radio-isotope assay similar to that reported by Parvin and Pande [18]. All serum samples were kept frozen at -80°C and thawed before assay. Total L-carnitine was determined on diluted serum samples that had been subjected to heat and alkaline pH to hydrolyse carnitine to acyl carnitine. Free L-carnitine was determined directly from untreated serum. Esterified carnitine was the difference between total carnitine and free carnitine. Each sample or hydrolysed sample was measured in triplicate and the result represented the average of the assay triplicates. The inter- and intra- assay coefficient of variation for both total and free carnitine are less than 5%.

DHEAS

Serum DHEAS was measured in triplicate by quantitative radioimmunoassay utilising a Coat-A-Count DHEAS assay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). All serum samples were kept frozen, and were thawed and diluted just before assay. All samples were evaluated simultaneously to minimize assay variability. The assay conditions used were those specified by the assay kit manufacturer. The inter- and intra-assay coefficients of variation of the assay are 7.5 and 7.4%, respectively. The detection limit of the assay, defined as the apparent concentration two standard deviations below the counts at maximum binding, was approximately $1.1\ \mu\text{g}/\text{dl}$.

Statistical analysis

Pearson correlation coefficients (r) were calculated to assess linear associations between pairs of continuous variables (different carnitine measures, DHEAS and age). As data were not normally distributed, the Wilcoxon rank-sum test with Bonferroni adjustment was used to test for relationship between sex, age and other continuous variables. Data analysis was conducted using SAS (SAS Institute, Cary, NC, USA) and Statview Software version 4.02 (Abacus Concepts, Berkeley, CA, USA). Significance was set at $P \leq 0.05$.

Results

Subjects

We obtained samples from 216 (85 men, 131 women) healthy adults aged 20–94.8 (mean 49.6) years. Racial distribution was as follows: 208 Caucasian, two Asian, two Hispanic, one Black and one Native American.

Correlation of carnitine and DHEAS in people

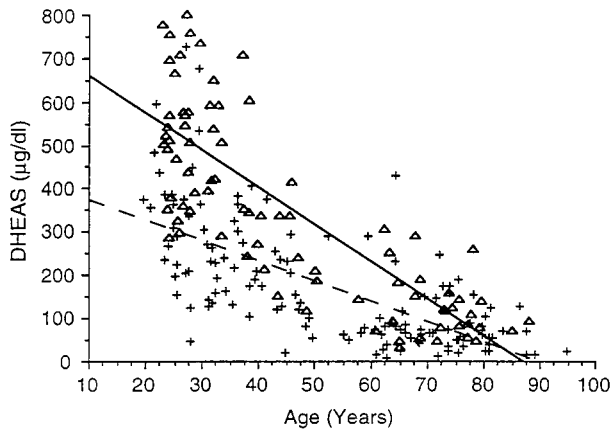


Figure 1. Correlation between serum dehydro-epiandrosterone sulphate (DHEAS) levels and age in women (+, -; $n = 131$) and men (Δ , -; $n = 85$). Women: $y = 422 - 4.7x$, $r = -0.677$, $P < 0.0001$; men: $y = 749 - 8.6x$, $r = -0.769$, $P < 0.0001$.

Table 1. Dehydro-epiandrosterone sulphate (DHEAS) levels by age and sex

	Mean DHEAS level (\pm SD), $\mu\text{g/dl}$		<i>P</i> -value ^a
	Young (20-60 years) ($n = 132$)	Old (>60 years) ($n = 84$)	
Male	466 \pm 192	126 \pm 77	<0.001
Female	252 \pm 145	83 \pm 72	<0.001
<i>P</i> -value	<0.001	0.024	

NS, not significant.

^aWilcoxon rank-sum test.

Age

Serum DHEAS levels declined with age in both the total population ($r = -0.693$, $P < 0.0001$, data not shown) and separately for each sex (men, $r = -0.769$, $P < 0.0001$; women, $r = -0.677$, $P < 0.0001$; Figure 1 and Table 1). Total ($r = 0.365$, $P < 0.0001$; Figure 2a), free ($r = 0.301$, $P < 0.0001$; Figure 2b) and esterified ($r = 0.368$, $P < 0.0001$; Figure 2c) carnitine levels and the esterified/free (E/F) carnitine ratio ($r = 0.173$, $P = 0.012$) increased with age. Using the rigorous threshold of an E/F ratio of ≥ 0.4 , 12% of our subjects had reduced serum carnitine availability. When examined by age group, 18% of our older individuals had a serum E/F ratio of ≥ 0.4 , whereas only 8% of the young subjects had E/F ratios of ≥ 0.4 .

Sex

Sex comparisons revealed lower total and free carnitine and lower DHEAS levels in women than in men (Table 2). A similar trend was evident for esterified carnitine (Table 2). For men, the decline in DHEAS

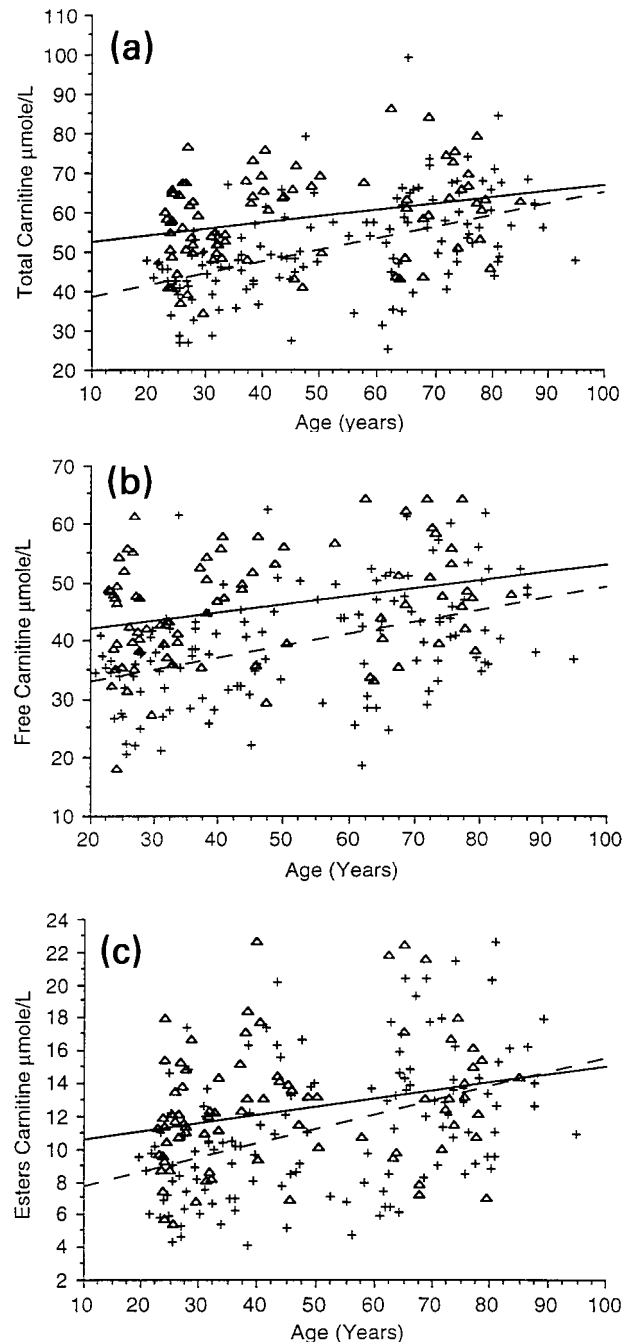


Figure 2. Correlation between serum **a** total, **b** free and **c** esterified carnitine levels and age in women (+, -; $n = 131$) and men (Δ , -; $n = 85$). **a** Women: $y = 36 + 0.3x$, $r = 0.499$, $P < 0.0001$; men: $y = 51 + 0.2x$, $r = 0.286$, $P = 0.007$. **b** Women: $y = 29 + 0.2x$, $r = 0.421$, $P < 0.0001$; men: $y = 39 + 0.1x$, $r = 0.298$, $P = 0.014$. **c** Women: $y = 6.8 + 0.09x$, $r = 0.438$, $P < 0.0001$; men: $y = 10 + 0.05x$, $r = 0.255$, $P = 0.012$.

level reached a plateau after the age of 60 years. There was a trend for women to have a higher E/F carnitine ratio than men (Tables 2 and 3). Young women had lower DHEAS levels than young men (Table 1).

Table 2. Total, free and esterified carnitine levels, ratios of esterified/free carnitine and dehydro-epiandrosterone sulphate (DHEAS) levels for combined ages

	Mean value (\pm SD)		<i>P</i> -value ^a
	Male (<i>n</i> = 85)	Female (<i>n</i> = 131)	
Carnitine (μ mole/l)			
Total	58 \pm 11	51 \pm 12	<0.0001
Free	46 \pm 9	40 \pm 10	<0.0001
Esterified	12 \pm 4	11 \pm 4	NS
Esterified/free carnitine	0.27 \pm 0.07	0.30 \pm 0.11	NS
DHEAS (μ g/dl)	355 \pm 230	176 \pm 145	0.0001

NS, not significant.

^aWilcoxon rank-sum test.

Table 3. Esterified/free (E/F) carnitine ratio by age and sex

	Mean E/F carnitine ratio (\pm SD)		<i>P</i> -value ^a
	Young (20–60 years) (<i>n</i> = 132)	Old (>60 years) (<i>n</i> = 84)	
Male	0.26 \pm 0.07	0.28 \pm 0.09	NS
Female	0.28 \pm 0.11	0.32 \pm 0.11	NS
All	0.27 \pm 0.09	0.31 \pm 0.10	0.007

NS, not significant.

^aWilcoxon rank-sum test.

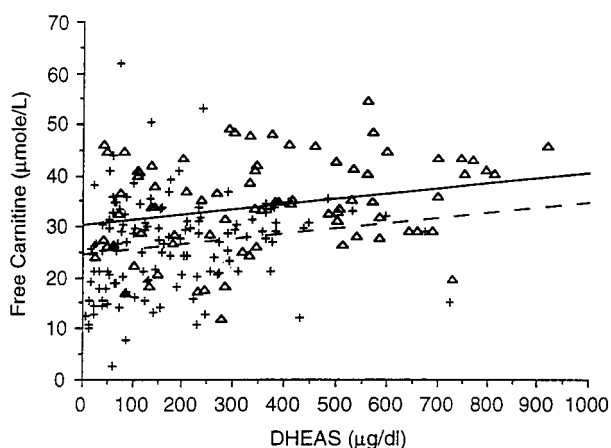


Figure 3. Correlation between age-corrected free carnitine levels and serum dehydro-epiandrosterone sulphate (DHEAS) in women (+, - -; *n* = 131) and men (Δ , —; *n* = 85). Free carnitine and DHEAS levels were correlated for each individual after correction for age using analysis of covariance. Women: $y = 25 + 0.01x$, $r = 0.161$, $P < 0.689$; men: $y = 30 + 0.01x$, $r = 0.258$, $P = 0.02$.

Correlation of DHEAS and carnitine

Free carnitine level was positively correlated with DHEAS in men after removing age-related variance. The E/F carnitine ratio declined with DHEAS levels for the whole population ($r = -0.215$, $P = 0.002$, data not shown). After correcting for age by analysis of covariance, the E/F carnitine ratio inversely correlated with DHEAS levels in men (Figure 3).

Discussion

In this study, we observed that carnitine and DHEA levels are related to age and sex. Like previous studies [19–21], we found that women had significantly lower total and free serum carnitine levels than men. Sex-specific differences in iron metabolism may account for this finding. Specifically, iron is necessary for the biosynthesis of carnitine [22]. That women have less iron than men [23, 24], either due to lower intake [21] or increased losses [25], could account for their lower carnitine levels. However, since iron stores were not assessed in this study, we were not able to establish a relation between this factor and our observations.

In contrast to previous studies that demonstrated increased serum total and free carnitine with age among only the women [4, 9, 10], our study identified increased total, free and esterified serum carnitine levels with age in both sexes. Our study population consisted of more subjects and a broader age range than the previous studies, which may account for our ability to detect an increase in these indices in men. In addition to the total and free carnitine levels, there was a similar increase in the E/F carnitine ratio with age in both sexes. The latter finding is of particular interest because it suggests that, in spite of the higher total carnitine levels accruing with age, there is relatively less free carnitine available for fatty acid oxidation and acyl group removal.

Carnitine deficiency has been variably defined using either low tissue [26], or serum levels of total carnitine [9]. However, some studies suggest that an increased E/F carnitine ratio may be the most relevant measure of carnitine status [7, 27–29]. In general, there is agreement that higher E/F ratios indicate greater relative unavailability of carnitine, ultimately causing carnitine deficiency. The increases in serum carnitine levels observed in our ageing population were due to a greater rise in esterified than free carnitine. These observations may be explained by factors known to occur during the ageing process. For example, the general decline in fatty acid oxidation that occurs with age [30] could result in decreased utilization of free carnitine, culminating in increased serum esterified carnitine [31, 32]. An alternative explanation is provided by the observation that during renal dysfunction there is a greater diminution of renal excretion of esterified carnitine than of free

carnitine [33–35]. Thus, it follows that as renal function declines with age, there is an overall increase in the E/F carnitine ratio. Finally, slowing or impairment of the cellular transport system for carnitine may result in elevated serum levels of free and esterified carnitine in aged individuals.

Our observation that DHEAS levels decline with age is consistent with previous reports [14, 36]. Reasons for the age-related decline in DHEAS and how sex affects this reduction are unclear. However, in women it may be related to menopausal status [36]. Additionally, decreased DHEAS biosynthesis secondary to declining adrenal function with age [37, 38] may account for the age-related decline of DHEAS.

In addition to DHEAS' ability directly to promote energy metabolism [39], our observation that carnitine levels correlated with DHEAS levels, independent of age, suggests that DHEAS can modulate carnitine levels. Although these data are correlative, they are consistent with the observation that DHEAS administration increases carnitine levels in rodents [40]. These results should be interpreted in the light cautiously as the nutritional status of our subjects was not investigated. Nutritional state can affect carnitine and DHEAS levels. However, these were mobile healthy individuals with no obvious evidence of malnourishment.

In summary, age-induced modulation of DHEAS and carnitine levels results in decreased carnitine availability. These findings may provide clues to understanding why mitochondrial function and energy metabolism decreases with ageing.

Key points

- Carnitine and dehydro-epiandrosterone sulphate (DHEAS) promote energy metabolism.
- Carnitine and DHEAS levels are lower in women than men.
- DHEAS and carnitine availability decline with age.
- Decreased carnitine and DHEAS levels may contribute to the decreased energy metabolism observed with ageing.

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