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Forum

Ascorbic acid, carnitine and fatigue

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This paper reviews the evidence that ascorbic acid (vitamin C) may have a contributory role in the prevention of fatigue in humans.

The biochemical history of carnitine (β-hydroxy-γ-*N*-trimethylammonium butyrate) is characterised by alternating periods of activity and dormancy. The re-emergence of interest during the past 15 years has been characterised by three main areas of emphasis — the endogenous biosynthesis of carnitine from lysine, the involvement of carnitine as a co-factor in the metabolism of long-chain fatty acids, and the identification of the comparatively rare, but metabolically interesting, carnitine deficiency diseases [1–4].

Biosynthesis of carnitine

Two essential amino acids — methionine and lysine — are involved in the biosynthesis of carnitine. Lysine is methylated to produce the protein-bound trimethyl-lysine which is then hydroxylated to form trimethylaminobutyrate (3-hydroxyltrimethyl-lysine); in a second hydroxylation step trimethylaminobutyrate (γ-butyrobetaine) is converted into carnitine [3]. These two hydroxylations are catalysed by separate ferrous-requiring oxygenases and both are stimulated *in vitro* by the addition of ascorbic acid; in the case of the trimethyl-lysine hydroxylase the ascorbic acid is partially replaceable by other reductants but the requirement of the γ-butyrobetaine hydroxylase for ascorbic acid would appear to be specific [5, 6].

More recently, the earlier *in vitro* work has been extended to whole animal studies. A number of reports have indicated that the formation of carnitine is impaired in guinea-pigs deprived of dietary ascorbic acid [7–10]. In perfusion studies it was shown that 'scorbutic' guinea-pig livers had a significantly reduced capacity for converting γ-butyrobetaine to carnitine and that this defect could be corrected by prior perfusion with ascorbic acid [11]. These and other aspects of the relationship between ascorbic acid and carnitine biosynthesis have been recently reviewed [12, 13]; England and Seifter [13] in their survey of the field concluded that there is *in vitro* stimulation of both hydroxylases by ascorbic acid, that there are variable decreases in tissue carnitine concentrations when guinea-pigs are deprived of ascorbic acid, and that the evidence, on balance, suggests that there is a reduced activity of liver γ-butyrobetaine hydroxylase in avitaminotic C guinea-pigs. It is difficult to quantify the amount (or proportion) of ascorbic acid required for the carnitine biosynthetic pathway. Dietary carnitine significantly prolonged the life span of scorbutic guinea-pigs, but only by some 10%, which would suggest that the requirement for ascorbic acid for carnitine biosynthesis is small when compared with its main metabolic role in the formation of collagen [14].

Metabolic role of carnitine

The essential role of carnitine in both man and animals is that it is required for the transport of long-chain fatty acids into the mitochondrial matrix where, by β-oxidation, they may be used as a source of energy [3]. Fatty acid-CoA esters are transesterified by carnitine acyl (palmitoyl)transferase I and the fatty acyl-carnitine esters are transported across the inner mitochondrial membrane by a carnitine-acyl translocase. Fatty acid-CoA esters are released by a carnitine acyl (palmitoyl)transferase II on the inner membrane and the cycle is repeated [3, 15]. A lack of carnitine, or a diminished or defective activity of one or more of the transport system enzymes, will reduce the availability of fatty acids as a source of energy. This, apart from producing a generally reduced 'energy status', could be of critical significance in situations where the bulk of the energy metabolism is believed to be derived from fatty acid metabolism — such as in the newly-born infant [16] or in the metabolism of normal cardiac muscle [17]. It has been suggested that endogenous production of carnitine is impaired in the newly-born infant and that consequently there is a requirement for a supply of dietary carnitine to maintain the necessary tissue concentrations. The rate-limiting factor in carnitine production is the ascorbic acid-dependent γ-butyrobetaine hydroxylase [18] and the ascorbic acid intake of a newly-born child would, of course, be low compared with that of a developing child on a mixed diet, as human milk contains only 3 mg ascorbic acid per 100 ml. However, in a recent publication, Olson and Rebouche [19] have indicated that γ-butyrobetaine hydroxylase is not rate-limiting for carnitine formation in the human infant.

Carnitine diseases

The identification and characterisation of the various carnitine diseases have produced useful information about the carnitine–energy relationship. Two types of primary carnitine deficiency have been recognised — the muscle or myopathic form which is largely confined to the skeletal muscle system, and a systemic form which is a more generalised disease [15]. The causes of carnitine deficiency in man are, theoretically, multiple and no single biochemical lesion has been defined. A common feature of the carnitine deficiencies is the progressively increasing muscle weakness and fatigue — a point of central importance to the main thesis of this review [20]. The clinical notes for the first reported case of systemic carnitine deficiency carry the observation "... a gradual development of muscle weakness ... he could no longer run ... he became short of breath after walking half a block ... he was always fatigued" [21]. Similarly, a 20 year-old male who presented with myopathic carnitine deficiency was "... able to walk only 100 yards on level ground and had great difficulty in climbing stairs ... there was generalised muscular weakness..." [22]. These descriptions bear a similarity to the clinical descriptions of the early features of scurvy (avitaminosis C) in man.

Fatigue in human scurvy

References to the early emergence in scurvy of fatigue and lassitude were invariable features of the earliest clinical descriptions of the disease [23]. Eugalenus in 1658 spoke of "spontaneous debility" [24], Lister, in 1696, wrote of "weakness of limbs and considerable fatigue" [25] and Sydenham in 1742 of "spontaneous lassitude and difficulty of breathing after exercise" [26]. Naval surgeons with first hand

experience of scurvy were equally clear in their descriptions: "The signes of the Scurvie are many, namely a general lazinesse ... shortnesse and difficultie of breathing, especially when they moove themselves" commented Woodall in 1639 [27] and Lind, over a century later, wrote: "... this lassitude, with a breathlessness upon motion, are observed to be among the most common concomitants of the distemper" [28]. Practising 'land physicians' in the last century made similar observations. Shapter, a careful clinical observer, describing an outbreak of scurvy in Exeter in 1847, perhaps put the matter most clearly: "... the spongy and swollen gum appears to me to have been erroneously estimated as amongst the primary and most obvious manifestations of the scurvy ... I am inclined to say there is a class of well-marked symptoms preceding this ... The first or initiatory stage ... has appeared to me to be characterised by ... debility ... weakness, listlessness and a disinclination to exercise" [29].

More recent cases of scurvy have also underlined the early emergence of fatigue. In 1952 it was noted in a case history that the patient had, during the year before admission, "become increasingly weak and easily fatigued" [30] and reports of experimentally induced scurvy in human volunteers similarly drew attention to the early emergence of fatigue [31–33]. Crandon, who placed himself on a scorbutogenic diet, commented that a feeling of fatigue developed from the beginning of the 3rd month of deficiency, a full 6 to 8 weeks before the emergence of the traditional 'overt' signs of scurvy such as perifollicular hyperkeratotic papules, petechiae, poor wound healing and softening of the gums [34].

It will be noted that the fatigue of scurvy, like the fall in muscle carnitine in hypovitaminotic C guinea-pigs [7], evidences itself before the traditional overt signs of scurvy and it has been suggested that it reflects an impairment of the endogenous biosynthesis of carnitine in the absence of adequate ascorbic acid [23]. The pathological features customarily associated with scurvy are all, theoretically, amenable to reductionist treatment in terms of the hydroxylation of lysyl and prolyl residues in the formation of collagen. Fatigue bears no identifiable relationship to collagen formation, and this is possibly the reason why this feature of incipient scurvy has been generally ignored by students of the disease.

Dietary significance

It has been estimated that over three quarters of the body carnitine in the adult rat and in the normal human is produced endogenously by the biosynthetic pathway from lysine and methionine [21, 35]. Vegetarians, with a negligible intake of preformed carnitine, maintain normal plasma concentrations [36] and by the same token, systemic carnitine deficiency is exacerbated by a vegetarian (i.e., a low carnitine) diet [37]. Any dietary influence on carnitine status in the normal adult is, therefore, most likely to be mediated via the availability of the precursor molecules and co-factors necessary for the biosynthesis. The most likely candidates in this respect are lysine (and, to a lesser extent, methionine) and ascorbic acid. (Theoretically, iron, nicotinic acid and pyridoxine could also be involved [2] but they fall outside the scope of this review and are, in any case, less likely to be limiting factors in the average diet).

There is evidence that modifications to the dietary lysine (or to lysine-rich proteins) result in changes in the carnitine status [38]. Carnitine formation is reduced by lysine- deficient diets [39] and urinary carnitine is decreased in persons receiving non-optimal diets with rice as the main component [40]. Adult Wistar rats receiving a diet to which protein contributed 31% of the total calories, excreted two and a half times as much free urinary carnitine as a corresponding group where the protein accounted for only 8.5% of the calories [41].

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In contrast to the influence of dietary protein on urinary carnitine in rats, changing the ascorbic acid status of guinea-pigs from hypovitaminosis C (produced by giving 0.2 mg ascorbic acid per 100 g body weight daily) to tissue saturation did not produce any resultant change in the excretion of carnitine [41] and in young and elderly women no relationship was found between plasma carnitine and leucocyte ascorbic acid although thirteen of the elderly women included in the study had a leucocyte ascorbic acid concentration below 15 μg/10 cells, customarily regarded as the 'scurvy risk' cut off point [42]. This would appear to suggest that protein is a more powerful determinant of carnitine biosynthesis than ascorbic acid. Nevertheless, there is some evidence that in man a relationship exists between ascorbic acid status and carnitine formation; in elderly men and women a highly significant positive correlation was found between urinary free carnitine and leucocyte ascorbic acid [42]. These are findings difficult to reconcile but there are indications both from accounts of carnitine deficiency diseases and from studies with normal adults that the relationship between serum carnitine and tissue carnitine concentrations is an equally erratic one [20, 43]. There is therefore no direct evidence that muscle carnitine in man is related to vitamin C status — and confounding factors such as dietary protein intake reduce the possibility of an easy resolution of the question. Nor does the plasma carnitine concentration present the type of correlation with age and sex found for ascorbic acid [44, 45].

Table 1: Ascorbic acid intake and self-assessed fatigue in adult females: combined results for two areas in Wales (means ± SEM) (S.I. Gruffudd and R.E. Hughes, 1988, unpublished results)

Ascorbic acid status	*n*	Mean ascorbic acid intake (mg/day)	Fatigue score
< 30 mg/day	51	19.1 ± 0.86	34.3 ± 7[a]
> 100 mg/day	54	142.6 ± 5.5	25.3 ± 2[b]
Random sample	76	69.9 ± 4.7	30.3 ± 1.0[c]

Significance of difference between mean fatigue scores: a and b, $p < 0.001$; a and c, $p < 0.05$; b and c, $p < 0.002$.

An alternative approach would be to examine the relationship between fatigue and ascorbic acid status by determining the degree of correlation between leucocyte ascorbic acid (generally accepted as an index of ascorbic acid status) and a measurement of physical fatigue. A less direct, and less satisfactory, method would be to substitute psychological self-assessment of fatigue for actual physical methods. Dietary-personality studies of this type have, hitherto, been little used in vitamin C work. In a recent preliminary study of this type the relationship between ascorbic acid intake (assessed by a frequency–portion size questionnaire) and self-assessed fatigue [46] was examined in 500 randomly selected adult females in two areas of Wales. There was a negative correlation between estimated ascorbic acid intake and the score for self-assessed fatigue. The fatigue score for intakes below 30 mg a day (the Recommended Daily Amount) was significantly greater than for intakes of over 100 mg (approaching tissue saturation) and both values were individually significantly different from a random sample of the total participants (Table 1).

Diet-personality studies of this type are of course fraught

with difficulties and are justifiably unpopular with nutritional scientists [47]; more than any other type of scientific exercise, they are open to spurious interpretations. The results of the study described above could be interpreted as implying that fatigue results in a more restricted choice of diet with a resultant reduction in vitamin C intake — instead of *vice versa*. The variables and imponderables present reduce the validity of a simple 'causal' explanation. Nevertheless, the general negative correlation found between ascorbic acid intake and fatigue score is consistent with three reasonably well-established findings: (i) ascorbic acid deficiency reduces the formation of carnitine; (ii) carnitine is necessary for the production of energy from fatty acids and impairment of this function results in fatigue;and (iii) fatigue is an invariable feature of incipient avitaminosis C (scurvy) in humans.

The current Recommended Daily Allowance (RDA) for ascorbic acid in the United Kingdom is 30 mg daily. This is based essentially on a single experiment done over 40 years ago on a small number of non-representative members of the population when the role of ascorbic acid was assessed solely in terms of the overt ('collagen defect') signs of scurvy [45]. The presence of fatigue, and its relationship to the ascorbic acid intake, was not studied. Both animal studies [7] and observations on human scurvy [23] appear to indicate that the maintenance of a functionally-appropriate level of carnitine and the prevention of physical fatigue may require an intake of ascorbic acid greater than that necessary to 'protect' against the traditional overt signs of scurvy. As the ascorbic acid intake of a substantial proportion of the population of the United Kingdom is currently below the RDA further studies on the relationship between ascorbic acid, carnitine and fatigue would be useful.

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