Review

Is a low concentration of Linoleic Acid related to the extended longevity of the Queen honeybee?

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Summary. Lifespan has been a topic of scientific interest for about a century and, in this regard, hundreds of theories have been expressed, calling into question numerous causes of aging, but none of which appears to be decisive. Different species can have hugely different species-specific life spans, but several eusocial species, e.g. honey bees, show very large differences in infra-specific longevity, that is, of individuals. One of the most widely accepted theory remains that of free radicals formulated by Dennis Harmann in 1956 in the historical work "**Aging: A Theory based on Free Radical and Radiation Chemistry**" (1**)** and in the following works, where the hypothesis foresees the existence of a strong metabolic activity in the production of free radicals, both on polyunsaturated fatty acids and on proteins with accumulation of oxidized products and damage at mitochondrial level. The effects of peroxidative damage seem to emerge as one of the factors most influencing lifespan. None of the works related to this longstanding problem, however, takes into consideration Linoleic Acid (C18: 2 n-6) as a central element among membrane fatty acids in the involvement of its peroxidation in relation to lifespan. In the examination of the main metabolic responsibilities of Linoleic Acid, attention is focused on the particular role that it, an essential fatty acid, could have as a determining element in the lifespan of worker honeybees with respect to the queen honeybee. **Highlights.** >Linoleic Acid is an essential fatty acid for mammalian life. >Linoleic Acid occupies a central position in the vital dynamics of animal and human organisms. >Linoleic Acid has the maximum "bulk" in the space within cellular membranes when compared to all the other polyunsaturated fatty acids. >High concentrations of Linoleic Acid can increase the risks of oxidation of Linoleic Acid itself by increasing the peroxidation index, more linked to Linoleic Acid rather than to other polyunsaturated fatty acids.

Key words: Royal bee, Worker bee, Lifespan, Linoleic Acid, Oxidation

Introduction

In trying understand the biological mechanisms at the basis of longevity, many animal models have been considered (2). Among these, the honeybee poses very difficult challenges to any aging theory. In general, social insects show a subdivision into fertile castes, females or queens and males or drones, and sterile one, or workers, and a division of tasks, in that the breeding caste focus exclusively on reproduction, while other tasks necessary for colony maintenance are performed by sterile caste.

Incidentally, evolution has produced a strong differentiation in longevity among the castes, reaching a lifespan of the queens by twice (ants) at tens of times longer than the workers (honeybees and termites). Worker honeybees, in addition, shift from age-dependent stages during their life, in which they develop relevant morphological changes in chronological succession and which are characterized by different aging rates. Nurse bees, who develop the hypopharyngeal glands for the production of royal jelly from day 5 to day 17, show a slow aging rate while foragers, in the last two weeks of their life, age rapidly (3). However, under specific environmental conditions, e.g. a noticeable increase in the inflow of nourishment or after any demographic change of the worker population, a part of the workers can shift bidirectionally to another stage. In particular, even the oldest foragers can revert to the feeding stage by developing the hypopharyngeal glands again, thus demonstrating that the transition from slower to faster aging is not inevitably linked to age (3). A further complication is given by the time of the season when the workers are born: the workers born in spring and summer have a maximum lifespan of about 65 days (4), while workers born at the beginning of the unfavourable season, which will not have to feed the larvae for several months because the queen's oviposition stops during winter, enter a "diutinus" stage (5), with the characteristic of nurse workers, and a prolonged life of up to 280 days . Against this variable, but still short life span of the workers, the queen normally lives up to 5 years.

Over more than a century of scientific studies, more than 300 theories on aging have been produced (6), but many of these could hardly justify how such plasticity could have been selected during evolution.

Aging theories can be roughly brought together under two main strands of thought, namely that according to which aging would be a genetically programmed event, which would manifest itself through changes in the functioning of the nervous, immune and endocrine systems, and that of the progressive accumulation of damage caused by the influence of the environment (7).

In this article, we discuss the role of linoleic acid as a key factor among oxidizing substances able of accumulating harmful free radicals at the level of cell membranes, with reference to the honeybee.

Linoleic Acid between regulation of biological membranes and pathology

Living systems are highly complex, expressive of a continuous relationship between the parts, in the sign of what Spinoza in Ethica called *conatus sese conservandi*. In practice, a persistence, an effort, a tension to keep oneself in existence, which coincides with a continuous expansion of power, energy: *posse existere potentia est* (Ethica, I, XI, sec.). We exist as a point of force, because we have power, albeit limited; we are dynamic forces that, by nature, tend to expand.

All this is of surprising beauty (8).

Of course, this power to exist send back to the duration of life: a power is such with respect to the quality and quantity of its expansion. Here, Linoleic acid plays an essential role.

Linoleic acid, an essential substance in the life of animal organisms, was discovered by Burr & Burr in 1929 (9) and Holman encodes his needs, for humans, between 1 and 2% of total calories (10).

The scientific works on Linoleic Acid are numerous, however this fatty acid is mostly studied in relation to health effects rather than biochemical and molecular effects.

C18:2 n-6 plays a key role in the cell membrane. It can regulate the osmosis of the cell, as occurs, e.g. in the epidermis and has a close relationship with cholesterol and oxygen (11).

Cholesterol appears to be involved in the removal of reactive oxygen substances from membrane phospholipids in order to prevent a harmful peroxidation effect of Linoleic Acid (12, 13).

Always from the molecular point of view, Linoleic Acid is involved in the functioning of ion pumps and ion channels of cardiomyocytes (14) and in the regulation of temperature where its concentration changes with respect to changes of the same temperature as it also happens in insects such as Drosophila (15) and in animals that live in extreme temperatures (16, 17).

The prevalence of studies on linoleic acid, on the clinical side, concerns the possible link with cholesterol, its involvement in atherosclerosis, its participation as a promoter in carcinogenesis, etc.

A careful examination of the literature, however, did not provide direct and / or certain evidence of this involvement (18).

In recent years, thanks to the results obtained from a research on ischemic heart disease and psychopathology (Major Depression and Bipolar Disorder), it has been sought to identify the position of Linoleic

Acid in its role as a mediator and conditioner of pathological conditions, precisely, as those mentioned above (19-24).

Other aspects have been analysed such as, for example, the relationship between Linoleic Acid and Cholesterol (25), where it is clear that due to their chemical structure, Linoleic Acid and Cholesterol, in biological membranes, must regulate themselves synchronously. That is to say, that if the Linoleic Acid reduces its concentration also the Cholesterol must reduce its concentration to guarantee the functional balance of the membrane, maintaining the right conditions of mobility (viscosity and fluidity).

Again, the hypothesis that there is a connection between Linoleic Acid and psychopathology passing through the concept of "symmetry breaking" (26) has been proposed, further, that Linoleic Acid may be the possible key that unlocks the quantum dimension of the brain (27).

In conclusion, an attempt was made to demonstrate that Linoleic Acid occupies a central position in the vital dynamics of animal and human organisms (28-30).

Linoleic Acid and Oxidation

Linoleic Acid has the maximum "bulk" in the space in which it is found (e.g.: cellular membranes), compared to all the other polyunsaturated fatty acids: this characteristic allows the approach of other chemical species with pro-oxidant activity to react with LA.

Furthermore, the structure of Linoleic Acid indicates that the enlargement of the sheets of the cellular phospholipid membrane, in which it may be inserted, will more easily cause a relaxation of the membrane itself, increasing its fluidity and, consequently, modifying its functionality. In these chemical-physical conditions of the cell membrane, it is easy to foresee an easier access by the Reactive Oxygen Species (ROS), but also a "recall" of balancing molecules to restore a correct membrane function. The cholesterol, particularly suitable for compacting the membrane, is transported to the cells by the LDL with a mechanism that will obtain a double effect: of rebalancing the molecules for the functionality of the membranes and removing the quantity of already oxidized Linoleic acid (LDL oxidized).Therefore, linoleic acid appears to occupy a central position in the oxidation-reduction balance of the cell.

The above mechanisms, in conditions of high concentrations of Linoleic Acid, can increase the risks of oxidation of Linoleic Acid itself by increasing the peroxidation index, more linked to Linoleic Acid rather than to other polyunsaturated fatty acids.

The mechanism of the described actions is expected to remain in equilibrium since, at the same time, LDL and cholesterol decrease in the plasma, however LDLox will inevitably be produced.

Most food sources containing Linoleic Acid and alpha Linolenic Acid (i.e. non-long chain PUFAs) are particularly rich in Linoleic Acid and quite poor in alpha Linolenic Acid. This means that the increased risk of cellular oxidative damage is mainly linked to the Linoleic Acid (31-35).

It has been shown that the greater the number of double bonds and less radical are formed in a watery environment (36).

The excessive intake of Linoleic Acid from food sources, therefore, can be considered at high risk of oxidation and can be involved in the progression of cellular aging.

Linoleic Acid: Queen Bee and Worker Bee, Pollen and Royal Jelly

Regarding the composition of fatty acids, pollen and royal jelly have very different characteristics.

Examination of the literature shows a fatty acid composition of the lipids of royal jelly with respect to pollen, which demonstrates how Linoleic Acid is present in pollen in very high concentrations (37). In fact the food material intended for the larva and for the whole life of the queen bee (royal jelly) is obtained by enzymatic processing of the pollen and honey (collected) by the bees of the "court", does not contain Linoleic Acid, since this molecule is transformed into short-chain lipid components characteristic of royal jelly (38). Linoleic acid appears to be the fatty acid that essentially makes the difference between the two foods. In light of the previously reported biochemical-molecular considerations, the aforementioned condition would seem to be of particular interest in order to avoid damage from peroxidation in the queen bee causing a dysfunction of its reproductive role (39).

Furthermore, the feeding of worker bees through the consumption of pollen and honey would justify the intake of Linoleic Acid in substantial concentrations (37).

Linoleic Acid and Royal Bee Lifespan: Hypothesis

The work of Haddad et al. opens to some interesting considerations about the relationship between the fatty acids composition of the queen bee compared to the working one as regards the possible cause of increase in the lifespan of the first compared to the second (40) and open an interesting discussion on the causes of aging of worker bees with respect to queens on the basis of the peroxidation index which characterizes the former with respect to the latter. This peroxidation index is three times higher in worker bees when compared to the queen bees. A careful reading of the data (fatty acids) contained in the aforementioned work also demonstrates that the fatty acid composition of the queens at birth and during aging is characterized by a substantial difference in concentration of Linoleic Acid in the queens compared to the workers and with respect to the alpha Linolenic Acid. The low concentration of Linoleic Acid in queen bee with respect to the worker bees is confirmed in all the districts (head, thorax and abdomen) analyzed (40). The very low concentration of Linoleic Acid, both, in royal jelly and in the body of the queen bee, seems to testify that the lack of Linoleic Acid protects the queen bee from the excess of peroxidation and that, consequently, increases the lifespan, unlike what happens in worker bees. In support of this hypothesis, it must be emphasized that the oxidation capacity of Linoleic Acid is greater than that of the alpha Linolenic acid, in an aqueous environment such as that of the cell membrane (41-43). If we observe the concentration of the alpha Linolenic Acid in the queen bees, it is higher than that of the Linoleic Acid, therefore it seems plausible the hypothesis that the oxidation of the alpha Linolenic Acid is indifferent with respect to the lifespan of the queen bee and that its lifespan depends mostly from the very low concentration of the more oxidizable Linoleic Acid. This observation, therefore, would justify the lower involvement of alpha Linolenic Acid in the determinism of the peroxidative phenomenon linked to aging. Further evidence regarding the Linoleic Acid is its concentration in the head (40) of the queen bee compared to the worker bee that is very similar to that found in the brain of mammal organisms (men and animals) living on earth, that is around 1% (44).

To better support our hypothesis, further studies are needed to verify if the trend pattern of the oleic acid content overlap significantly on the 3 female phenotypes characterized by as many physiological stages, namely the nurs, diutinus and forager workers (3). These stages are reversible between them and age-independent and therefore cannot be compared with workers schematically collected at a certain age (40) but need to be well characterized in the experiments.

It is also necessary to correlate the body's oleic acid (less susceptible to oxidation) (45) content of the different phenotypes with the relative amounts of royal jelly, pollen and honey fed to the different phenotypes. In fact, the relationship between the two foods in the daily diet of workers also varies in relation to the phenotypic stage.

Conclusion

In light of the acquisitions on the functional characteristics of Linoleic Acid and on its greater facility in oxidizing compared to all other polyunsaturated fatty acids, the consistent reduced concentration of Linoleic Acid in queen bee with respect to the worker bee, can allow the hypothesis of an increased oxidative phenomenon in the worker bee with consequent effect on the life span.

In the honey bee female eggs become workers or queens, depending on what they are fed, without any apparent difference in the common genome. If further research were to confirm there is a link between titer in linoleic acid and infraspecific longevity difference, and that this depends on differentiated composition of fatty acids in the nourishment, this would be a point in favor of the theory of progressive accumulation of damage caused by the influence of the environment. However, considering that nutrition is differentiated starting from the egg stage, it is difficult to understand such an impressive effect in terms of physical and physiological changes without imagining that there are switches in the genome capable of turning on the expression of a particular program following a food stimulus (46).

For this reason, we believe that social insects, and in particular the honeybee, a farmed species, can be an excellent model for the study of the factors that guide the evolution of longevity in species.

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