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ORIGINAL INVESTIGATION

Implementation of the Dutch Meeting Centres Support Program for people with dementia and their carers in Milan: process evaluation of the preparation phase

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Background and Aims. The Meeting Centres Support Programme (MCSP) for people with mild to moderately severe dementia and their carers proved effective in the Netherlands, and is now being implemented in other European countries. This study aimed to compare factors that at the preparation of two meeting centres in Italy were expected and experienced as facilitating or impeding implementation.
Methods. At the start, stakeholders (n = 19) filled in a checklist on expected facilitators and barriers. After opening the centres, experienced facilitators and barriers were inventoried in semi-structured interviews (n = 13) and analysed by two independent researchers, using a Theoretical model on implementation. Additionally, minutes of the initiative group were investigated. Expected and experienced facilitators and barriers were compared.
Results. In contrary to the expectations, the use of existing networks/collaboration between organizations facilitated the preparation phase. As expected, motivated stakeholders were facilitating. Shortening of the original time plan (for pragmatic reasons) was not expected, and made some preparatory tasks difficult to fulfil. Lack of Italian examples and cultural differences in working method made the realization of meeting centres difficult to imagine. Some experienced factors were not foreseen due to unexpected events.
Conclusions. Most aspects of MCSP appeared well implementable in the Italian setting. Many factors were in line with the Dutch implementation study, new influencing factors were also found.

Key words: Meeting centres support programme, Adaptive implementation, Dementia, Carers, Facilitators and barriers

INTRODUCTION

Worldwide, the number of elderly people (aged 60 years and older) with dementia is expected to increase from 46.8 million in 2015, to approximately 131.5 million in 2050 [1]. Research has shown that people with dementia and their informal caregivers, who are mostly family members (hereinafter carers), are facing numerous difficulties [2-4]. For example, people with dementia may have fears and ambivalent feelings regarding the dementia diagnosis. Because of that and of anosognosia tied to the neurodegenerative illness itself they may deny their symptoms and postpone asking for help in the early stages of the disease [4 5]. Likewise, Tremont [2] found that carers may find it difficult to cope with changing care demands and unexpected problems. Some carers may feel that asking for help is a sign of incompetence to cope with these difficulties, and consequently do not ask for help, especially not in the early stages of dementia [3]. However, waiting with asking for help until

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later stages, can result in accelerated nursing home admission of the person with dementia [6]. Furthermore, it can cause mental health problems and result in overburdening of carers [2 3].

To support either the person with dementia or the carer, numerous Single-component Support Programmes (SSP) are available. Examples of SSP for people with dementia are: psychosocial interventions, such as psychomotor therapy, memory groups and cognitive stimulation therapy [6 7]. SSP for carers are: support groups and educational programmes [6 7]. However, many of these SSP do not meet all individual needs and preferences of people with dementia and carers [9]. In contrast, Combined Personalizable Multi-component Support Programmes (CPMSP) offer supportive activities to both the persons with dementia and carers, adjusted to their multiple needs and preferences. CPMSP appear more effective than SSP: the general mental health of both carers and people with dementia is improved and admission to nursing homes is delayed [9 10].

An example of a CPMSP is the Meeting Centres Support Programme (MCSP) which was developed more than 20 years ago in the Netherlands. The MCSP is build on a theoretical framework, the Adaptation-Coping model [12]. The programme is meant for people with mild to moderately severe dementia (Global Deterioration Scale-score 4-6) who do not have severe behavioural problems (such as wandering) or movement problems and live at home, and their carers [13]. The MCSP consists of a social club for persons with dementia, which is generally accessible three days a week, where they can participate in recreational and therapeutic activities, such as billiard, cognitive stimulation therapy, creative art, and psychomotor therapy. Furthermore, support is offered to their carers by discussion groups and informative meetings. The staff also helps to coordinate care at home. Also, both people with dementia and carers can participate in social activities, a weekly consultation hour and regular centre meetings in which participants, employees and volunteers share experiences and discuss the support programme. All these supportive activities are adapted to both the people with dementia's and carers' needs and preferences [14 15].

The Dutch MCSP was compared to psycho-geriatric day care organized in nursing homes, and the effects were in accordance with the positive results which were found in other CPMSP [14 16-18]. People with dementia participating in a MCSP showed less mood and behavioural problems and a higher self esteem, and admission to residential care was delayed compared with those receiving regular psychogeriatric day care [14 16 17]. Carers felt less burdened and more competent, while lonely carers had fewer psychosomatic complaints [16 18]. Currently the MCSP is implemented in 144 meeting centres in the Netherlands, but this implementation did not occur spontaneously. According to Grol and Grimshaw [18], there is still a gap between scientific evidence of innovations and the actual implementation of these innovations in practice. Therefore, Grol [19] proposes a cyclical model which can be used to improve the implementation of care innovations. The model emphasises the importance of identifying obstacles to change and linking these obstacles to the implementation of the intervention. This idea is confirmed by, Meiland, Dröes, De Lange & Vernooij-Dassen [20 21] who have demonstrated that for a successful implementation of MCSP in the Netherlands, effective implementation strategies which take into account impeding and facilitating factors at various levels (micro, meso, macro) and in various phases (preparation, execution, continuation), are important. Several of these factors include: enthusiastic and active initiators, assessing the need for a meeting centre in the region, finding an accessible social integrated location, collaboration between welfare and care organizations, recruiting funding organizations, and awareness of the meeting centre pioneers about laws and regulations.

Within the framework of a joint European Programme (Joint Programme Neurodegenerative Diseases) the Dutch MCSP is further disseminated and adaptively implemented in three European countries, Italy, Poland and United Kingdom, in the MEETINGDEM project (www.meetingdem.eu). In Milan, Italy, two centres were planned to open in September 2015. These centres were adaptively implemented, taking into account characteristics of the local situation. Local situations between and within countries may differ. For example, the local situation in Milan may differ from local situations in The Netherlands: there are for example differences in organization of healthcare [19]. The Italian healthcare system is mainly based on the Beveridge model and the Dutch on the Bismarck model [20]. Because of these differences, other implementation strategies may be needed in Italy, than in the Netherlands. MCSP was implemented for the first time in Italy. No previous study investigated the implementation process and the used implementation strategies, specifically in an Italian setting. Gaining insight into aspects of the adaptive implementation process is considered to be an important step for successful implementation and further dissemination in Italy and Europe.

This research describes the preparation phase of the implementation of MCSP in two meeting centres in Milan, with the aim of investigating the factors that facilitated and/or impeded this implementation. Special attention is given to possible discrepancies between expected facilitators and barriers on beforehand and those actually experienced during the preparation phase.

MATERIALS AND METHODS

Design

A qualitative descriptive study was conducted in which stakeholders' expectations of facilitators and barriers at the start of the implementation of MCSP were compared with actual experienced facilitators and barriers during the preparation phase. Approval of the Medical Ethical Committee (MEC) was not required for this process analysis as there were no patients involved [21].

Study population and setting

All stakeholders present at the first initiative group (IG) meeting (n = 19), filled in a checklist about foreseen facilitators and barriers. The IG is a multidisciplinary group of representatives of relevant local care and welfare organizations, who were involved in the implementation of the MCSP in Milan. At the end of the preparation phase, stakeholders (n = 13) were interviewed about the experienced facilitators and barriers. These stakeholders were purposively selected based on their role in the project and their profession, ten of them were members of the IG (Table I). The IG was divided into four smaller subgroups with four to five members. Each subgroup worked on one or multiple preparatory tasks and reported results in the full IG: subgroup I worked on the definition of the target group, subgroup II defined the support programme, subgroup III elaborated on the location requirements and financing, and subgroup IV focused on the personnel/volunteers (tasks, function requirements, training). Other interviewed stakeholders were the two project managers of the MEETINGDEM project in Italy, who planned and organized the project and led the IG meetings. Furthermore, both coordinators of the two meeting centres (of which one was also member of the IG) were interviewed.
This study was carried out between March 2015 and September 2015. Both meeting centres opened, during the course of the study in May 2015 and were situated in community centres for elderly people, in zone 4 and 7 of Milan.

Data collection methods

The data collection methods consisted of administration of questionnaires and semi-structured interviews, and the collection of relevant documents regarding the implementation process. The questionnaire was used to inventory expected facilitators and barriers of implementation of MCSP. This questionnaire was based on the literature about impeding and facilitating factors of implementation of MCSP [22 23] and the theoretical model of Meiland et al. (Table II) [24] to trace facilitators and barriers of implementation of care innovations. In this model two types of facilitating and impeding factors are distinguished: 1) factors related to preconditions at the start of the implementation and/or during the whole implementation process and 2) factors which are specific for the different phases of implementation, in this study limited to the preparation phase. Preconditions are: characteristics of MCSP, time available for the implementation and operational preconditions, human and financial resources and organizational conditions.

Table I. Characteristics of interviewed stakeholders (n = 13*).

Role in MCSP project (number of stakeholders)	Profession (years of experience)	Age (sex)
IG subgroup I (n = 1) Definition of target group	Neurologist in hospital (19)	56 (f)
IG subgroup II (n = 4) Definition of the support programme	Responsible of hospital volunteer association (10) Volunteer of hospital volunteer association (17) Physiotherapist in cognitive rehabilitation & counsellor of Alzheimer association (17)* Psychologist alzheimer federation (9)	49 (f) 56 (f) 41 (f) 33 (f)
IG subgroup III (n = 2) Location requirements and Financing	Official of social services Municipality (40) District director of social-sanitary organization (36)	60 (m) 60 (f)
IG subgroup IV (n = 3) Personnel/volunteers	President Alzheimer association (30) Psychologist Alzheimer association (2 5) Psychologist in care/welfare cooperation (2)	61 (f) 26 (f) 30 (f)
Project managers (n = 2)	Psychologist clinical and research in hospital setting (7) Neurologist clinical and research setting in hospital (23)	33 (f) 52 (f)
Coordinators Meeting Centre (n = 2)	Physiotherapist in cognitive rehabilitation & counsellor of Alzheimer association (17)* Coordinator of elderly day-care (10)**	41 (f) 46 (f)

* This stakeholder was involved in IG subgroup II and was also a coordinator of one meeting centre, and is listed twice
** This stakeholder did not fill in the checklist
f = female
m = male

Table II. Preconditions and factors of the preparation phase from the 'Theoretical model to trace facilitating and impeding factors of implementation' of Meiland et al. [24].

1) Preconditions	2) Factors of the preparation phase
Characteristics of MCSP Time and operational conditions Human and financial resources Organizational conditions	Micro level (individual/meeting centre level) Meso level (organizational collaboration level) Macro level (healthcare system, legislation and policy level)

Factors specific for the preparation phase are distinguished in three levels: micro level (individual user, personnel, meeting centre level), meso level (organizational and collaboration level) and macro level (healthcare system, legislation and policy level).

The semi-structured interviews were led by a topic guide which was based on this theoretical model [24], available documents about the project and literature about impeding and facilitating factors of implementation [22 23 25]. Furthermore, regarding to the stakeholders' role in the preparation phase and their area of expertise, different aspects were discussed into more detail. Topics which were not described in the topic guide, but which were mentioned by the stakeholders were also discussed. In this way, the importance of each topic was determined by the information which was given by the participants [26].

Relevant documents that were collected were minutes of the meetings of the Initiative Group and results of the work in its subgroups.

Procedure

The main researcher (MS, Msc Health Sciences Graduate, sufficient knowledge of the Italian language, female) approached all stakeholders by telephone or e-mail with help of the two project managers (EF, neurologist and FS, psychologist), explaining the aim and scope of the research. All contacted stakeholders agreed to participate. They received an information letter and signed a letter of informed consent.

In total eleven semi-structured interviews were conducted with thirteen stakeholders in Italian by MS, principally at the workplace of the stakeholder. Two of the interviews concerned duo interviews, since in both cases a second stakeholder expressed her willingness to participate in the research. After each interview, a methodological memo was written about the execution of the interview.

Privacy of all participants was ensured by replacing private information (i.e. names) with codes. The key to these codes was maintained in a secured safe. The interviews lasted between 26 and 73 minutes (mean duration of 55 minutes), and were transcribed verbatim with Express Scribe Transcription (http://www.nch.com.au/scribe/) by MS.

Data analysis

Two independent researchers MS and RC (Associate Professor of clinical psychology, male), analyzed the transcripts, combining a deductive and inductive method [27]. First, the Italian transcripts were re-read and an English summary was made by MS. Each transcript was separately (deductively) analyzed in Italian by MS and RC in Excel, by making use of the theoretical model of Meiland et al. [24]. New codes were added where necessary (inductive). All codes and their definitions were discussed between the researchers until consensus was reached. Next, MS organized the facilitating and impeding factors in a coding tree with the use of Mindmap (https://www.xmind.net/xmind6/). Per code the fragments of different interviews were collected, which enabled to search for similarities and differences between the interviews. The main findings on facilitators and barriers of the implementation were described (MS) in a short summary and sent to the stakeholders, none of the stakeholders had any comments or extra information to add. Subsequently, MS compared the experienced facilitating and impeding factors with those expected on beforehand in the questionnaire. Meeting minutes of the IG meetings were used to get a better understanding of the implementation process of this preparation phase, including the differences found between the expected and actually experienced facilitators and barriers of implementation. Finally, a description was made of the whole preparation process, and of the factors that had facilitated or impeded this phase of implementation.

RESULTS

Preparation process of the MCSP implementation

May 2014, all relevant organizations in the Milan area were invited to an information meeting. June 2014, the first IG meeting took place in which an overview of the whole project and the preparation phase was given. At this meeting four subgroups were formed. Each subgroup was dedicated to specific tasks, related to the preparation of the implementation of the meeting

centres. These concerned: the target group, the support programme, the location and financing, and personnel. The tasks "communication plan/PR" and "protocol for collaboration" were not specifically allocated to subgroups. Furthermore, in this first meeting the members of the IG filled in a questionnaire (hereinafter checklist) on expected facilitating and impeding factors, and rated the importance of each of these factors (minor, intermediate, major) (Table III). September 2014, in the second meeting the facilitators and barriers were further discussed and possible solutions were identified and discussed. From September 2014 onwards, there was a monthly IG meeting, in which each subgroup worked separately on their own topic. The scheduled time for the actual preparation phase was approximately 16 months, however due to pragmatic reasons (see below result section) this had to be shortened to 12 months. The 5th of May 2015 the first meeting centre opened in Milan, and the second opened the 25th of May 2015. After the opening, the IG was transformed into an advisory committee, however not all members of the IG participated.

Expected and experienced facilitating and impeding factors

For each of the existing preconditions and factors specific to the preparation phase the main expected and experienced facilitators and barriers are described. The main experienced factors are presented in *Italic*. A complete overview of the factors that facilitated or impeded the preparation of the implementation of the meeting centres is shown in Table III.

Preconditions

Characteristics of MCSP

In the checklist on expected facilitators and barriers, the stakeholders described they believed this program would have a surplus value for people with dementia and carers compared with other programs for this target group. During the preparation phase, the people with dementia and carers could not yet really experience this surplus value, since the meeting centre was not yet opened. Nevertheless, the stakeholders felt there was a *need for this type of innovative program* in the Milan area, offering *integrated support to people with mild to moderately severe dementia and their carers three days per week*. As one of the stakeholders of the meeting centres described:

There was for example a relative, who said to me: "I was waiting for a place like this, where I can talk with a psychologist, but also my wife can do an activity here. There is also a lawyer, in case I have problems related to the administration, or to legal things, I can ask information. So, without having to go to all services of the city". (R8)

According to the stakeholders, current services were often fragmented, not frequently accessible, and few dementia services were available for this specific target group. They explained that the surplus value of the program compared to existing services amongst other things, motivated them to be involved in the program. Furthermore, the IG expected that studying existing examples of meeting centres would help them adapt the programme to the local situation, as they indicated in the checklist. During the preparation phase they experienced, that the *translated Meeting centres guide* on existing Dutch meeting centres was indeed useful and helped in guiding the work on the project. However, as the project was new for the stakeholders and *no Italian examples of meeting centres* were available at that time, in this initial phase some stakeholders found it difficult to imagine how the project could be realized. That is why some stakeholders suggested during the interview, that in order to further facilitate the implementation it would have been better to study the Dutch examples in even more detail in the initial phase, to help them understand what aspects of the MCSP could be used in the Italian setting without changes and what needed to be adapted. As a stakeholder explained:

"I would do something, not really starting from zero, but I would say that this are the experiences, let's say MCSP in the Netherlands functions like this. And you, how would you do it here in Milan? A greater reference to already implemented models. You always learn from the experiences of others". (R5)

Time and operational preconditions

In the checklist, the stakeholders emphasized the necessity of having enough time for the preparation phase, in order to explore the opportunities and resources available in the region as well as to enlarge networks. At the start of the project, they expected to have enough time to execute these preparatory tasks. However, the centres had to open four months earlier in order to obtain necessary resources, offered by the Municipality of Milan. Due to this, especially stakeholders with a major role in the project, such as the project managers and the coordinators of the centres, experienced a *lack of time* and a high workload. According to them, this factor had indeed a great influence on the preparation phase: some preparatory tasks were not done (e.g. signing collaboration protocol), some were not executed as planned (e.g. informal instead of formal needs assessment for a MCSP in the selected districts) and some were postponed to the execution

Table III. Expected and experienced impeding and facilitating factors, according to the stakeholders.

Theme's	Expected factors	Experienced factors
Preconditions: 1. Characteristics of MCSP	Surplus value for patient as well as carer (MAJOR) (=) Costs and surplus beneficial effects (MAJOR) (N.A.) Project attuned to needs, wishes, values in Milan (MAJOR) (») Examples of previous services available and availability of a course for personnel (INTERMEDIATE) (») Scientific research embedding could facilitate tasks of the IG (INTERMEDIATE) (»)	*Innovative centre on territory of Milan: need for integrated support for both people with mild to moderately severe dementia and their carers.* *Frequently accessible Meeting centres: motivated IG members to be involved.* *Existing European project guide gave direction to the work.* Involvement care/welfare territory of Milan helped adapting to local circumstances. Experimental status of project motivated IG members to be involved.
	No competition of MCSP with other initiatives could lead to less incentives to improve the project (MINOR) (N.A.)	*No Italian examples available: difficult to imagine how the project would evolve.* *Complexity of the project made it difficult to understand how it could be realized.* Taboo on dementia: was thought to impede recruitment of people with dementia and acceptability of the project.
Preconditions: 2. Time	Enough time will be needed to explore possibilities and perform the tasks of the IG (MAJOR) (X)	Right timing for open centre to maintain enthusiasm
		Lack of time: impeded some preparatory tasks of the stakeholders with a major role, in multiple ways.
Preconditions: 3. Human and financial resources	Competent project manager and a transparent project plan (MAJOR) (»)	*Project managers: motivated IG members to collaborate, were committed to invest time and possessed organization skills.* *Coordinators of the centre's: motivated personnel and volunteers and were committed to invest time in preparing the centre.* Personnel/volunteers: were qualified, motivated and experienced with dementia.
	Financial resources/ organizational structures available will be crucial for the development of the project but difficult to obtain (MAJOR) (=)	
Preconditions: 4. Organizational conditions	Enthusiasm of involved parties could motivate participants and give them new idea's (INTERMEDIATE) (=)	*Existing networks and collaborations: helped involving relevant actors to IG, helped obtaining resources and facilitated collaborations in the project.* Persons involved with knowledge of laws and regulations.
	Active role in region could promote the dissemination of the project, but is not considered to be fundamental (MINOR) (N.A.) Existing dementia care networks could help to integrate the project, but it is not necessary (MINOR) (»)	*Organizations are fragmentized on the territory of Milan: impeded involvement of some actors to the IG and impeded the collaboration in the IG.* Regional differences in Health care organizations requires local adaptations.
Factors of the preparation phase: 1. Micro level	Heterogeneous group of personnel/ volunteers could give a richer view (MAJOR) (=) Preparation of the location could help to think about concrete aspects of the project (INTERMEDIATE) (=)	*Most members of IG were motivated to invest a lot of time.* *Members of IG: experienced, competent, educated and professional.* *Two free and adapted locations were offered by the Municipality of Milan.* Several organizations offered free personnel and volunteers. Each member of IG spread information about the project and recruited participants. Geographical proximity of the meeting centre to organization involved in the project, facilitated recruitment of personnel/volunteers and people with dementia.
	Presence of enthusiasm among project members could be facilitating, the absence could lead to working in a hasty way (MAJOR) (=) PR strategies/informative meetings could be energy and time consuming in the initial phase, but it is considered to be important in an advanced phase (MINOR) (»)	*Difficult to find a suitable location: due to budget constraints and established criteria.* Reduction in opening hours of the centre: was thought to impede participation of carers. Difficult to find suitable personnel/volunteers: due to lack of time and professional requirements. Difficult to spread information about the project in a structured way, due to lack of time. Materials for centre lacking at opening of the centres Insufficient communication with potential participants (people with dementia and carers) was thought to lead to confusion with other services, in the future. Difficult collaboration with other users of the location impeded the preparation of one centre.

▶

Theme's	Expected factors	Experienced factors
Factors of the preparation phase: 2. Meso level	Initiative group (composition of heterogonous subgroups, frequency, division of responsibilities) could facilitate the preparatory tasks (INTERMEDIATE) (=) Responsible managers could serve as contact persons for the IG members (INTERMEDIATE) (=)	*Working in subgroups permitted to work in a focused way.* Will to add innovate activities in order to optimize the activity program. Project was supported by the Alzheimer network in Milan. Communication between subgroups was needed to harmonize the work. Planning of monthly meetings guided the work of the IG members.
	Financial support: difficult to understand from where it could be obtained (MAJOR) (=)	*Difficulties regarding the working method, impeded the preparatory tasks of the stakeholders, in multiple ways.* Insufficient communication in the big IG gave some members wrong or inadequate ideas about the progress of the project.
	Collaboration with organisations outside dementia care could be energy/time consuming in the preparation phase. Collaboration with organizations involved in dementia care is considered important from the start of the project. (INTERMEDIATE) (»)	*Heterogeneous IG, gave a more complete view, but also caused conflicting ideas.* *Checklist: difficult to fill in, but helped to think about implementation of certain aspects of the program.*
Factors of the preparation phase: 3. Macro level	Health insurance regulations and norms are easy to manage if there is one person with knowledge and difficult if knowledge is scattered among members (INTERMEDIATE) (=) Support of national parties: it would be helpful to have a national governmental plan on dementia (approved end 2014) (MINOR) (N.A.)	Laws/regulations issues were facilitated by persons with sufficient knowledge and availability of adapted locations.
		Difficulties in obtaining financing due to Italian Health care framework. Insecurity about how to finance the meeting centres support programme in the future.

+ Factor is expected/experienced to facilitate the implementation, - Factor is expected/experienced to impede the implementation and ± Factor is expected/experienced to facilitate and impede the implementation.
(MINOR): factor is expected to have a minor impact on the implementation; (INTERMEDIATE): factor is expected to have an intermediate impact on the implementation and (MAJOR): factor is expected to have a major impact on the implementation.
(=): factor is experienced as expected, (»): factors is partially experienced as expected; (X): factor is not experienced as expected and (N.A.): not applicable, not experienced yet. Written in *Italic:* the main experienced factors, which are described in detail in this section.

phase (necessary preparations for the functioning of the centres, such as obtaining materials and developing a network in the district). As one stakeholder explains:

"Also the network of relationships in the district. Right now [execution phase, MS] exactly, I am contacting the Local Health Centres and various centres for elderly. It is something, that probably had to be done a bit before. But there was no time". (R4)

Even so, generally the stakeholders indicated to be satisfied with the work performed in this short amount of time. Additionally, they considered it to be the *right moment to open the centres*, in order to profit from the existing enthusiasm of all involved in the project.

Human and financial resources
The stakeholders expected it to be important for the project to have a project manager able to coordinate preparatory tasks. During the preparation phase, the two *project managers* stated to be very motivated/enthusiastic and they invested a lot of their time in the project in order to finish the work properly. According to other stakeholders they organized the project very well and they guided them in their work. A member of subgroup IV (personnel/volunteers) commented on this as follows:

"The project managers are two really super competent persons. Well, it is their competence obviously which was essential for the realization of this project, obviously their commitment, their competences". (R7)

Also the *coordinators of the meeting centres* stated to be very motivated/enthusiastic and they invested a lot more time then the prescribed nine working hours per week. They motivated the personnel/volunteers, for example by organizing a meeting before the centre opened in order to get to know them.
The financial resources are discussed under the subheading macro level.

Organizational conditions
At the start, the stakeholders emphasized in the checklist the importance of collaborating with organizations involved in dementia care, while they considered the

collaboration with organizations *not* involved in dementia care to be too energy and time consuming, especially in the preparation phase. In addition, the use of existing networks was expected to be helpful but not necessary to develop the project. However, during the preparation phase, the stakeholders experienced that the use of *existing networks and collaborations* with both types of organizations was very important. The main resources were obtained by using the IG members network, such as the two financing organizations (Municipality of Milan and a Swiss foundation), which also offered locations and personnel. As one of the stakeholders explained:

"A key element was the inclusion of a member of the municipality in the project. She was not included immediately, but she was included thanks to a suggestion by one of the members that came to the first initiative group meeting". (R10)

In addition, existing collaborations between organizations facilitated other collaborations between organizations in the project. For example, the municipality had an existing collaboration with other users of the proposed location, this improved the willingness of these users to collaborate with the MCSP.
Before the project started, collaborations between different care and welfare *organizations in the Milan area* often did not exist, they were very fragmented and had different visions. Because of this, the project manager found it difficult to identify and involve the relevant actors in the Milan area to the IG. Therefore, some stakeholders missed the first IG meeting.

Preparation phase

Micro level
The stakeholders expected that the presence of enthusiasm among them would facilitate the preparatory tasks. They felt that absence of enthusiasm would make them execute the work in an impetuous, quickly and hasty way. During the preparation phase many stakeholders experienced that the *IG members were indeed very motivated* to do something good for people with dementia and therefore they invested a lot of time in the project, mainly for free. However, not all members were able to attend all meetings. One member of subgroup II (support programme) described:

"Well, being there all together for a common purpose, that of being able to improve, wherever possible, the quality of life of the ill person and his family. That was, according to me, the thing which united us a bit, which made it a success, that everybody was there for a single purpose". (R6)

They considered the *members of the IG* to be experienced in the field of dementia, educated, competent and professional. Because of that, they knew what people with dementia and carers needed, had many ideas, and could therefore contribute much to the project.
In the checklist, the stakeholders described that the preparation of the location was expected to help them think about the practical aspects of the project. Once the location was found, the project did indeed become more concrete to several stakeholders. However, they found it difficult to find a suitable location, because the *choice of location was restricted* by the available budget and by the established criteria for the centre. Eventually, two easily accessible locations were *offered for free by the municipality of Milan*. In one of the centres there was a good *collaboration with the other users of the location*, because they were already interested in the topic of dementia and therefore they were also interested in the project. However, in the second centre the relationship with other users of the location was difficult and impeded the preparation phase: According to the stakeholders these other users were not interested in the topic of dementia, it might not have been clear to the other users what was needed in order to realize this project and the other users believed that in the end the centre would not be realized. This difficult collaboration delayed the opening of the second centre with three weeks. The stakeholders did not expect to have such a difficult relation with these other users of the centre, as one stakeholder explained:

"A very big difficulty what we had not expected was the hostility by a certain part of the population to the project, because for example in district (…) we have these type of problems". (R10)

Meso level
The stakeholders expected that the IG would profit from the division in smaller heterogeneous subgroups, especially after two to three meetings. They indeed experienced during the preparation phase that the *heterogeneous IG* gave a more complete overview and competences of the single members were complementary. Only sometimes the different backgrounds and different views seemed to impede the work. As one member of subgroup IV (personnel/volunteers) explained:

"Many different experiences, it's not easy to make the people work together at the same moment, but in the end you are able to develop a much richer proposal". (R3)

In addition, the work of the IG was also experienced to be facilitated by the *creation of subgroups*, each

subgroup was able to focus on one topic without being dispersive.
The stakeholders explained that some aspects of the *working methodology* of the European project were not clear and/or not suitable in the Italian context. For example, initially some of them thought they had to develop the project, which was not the case. In the advanced phase of the preparation, others had preferred to follow the European working methodology of the project less strictly, and to adapt the program more to their own ideas. Furthermore, some explained that they "Italians" were not used to think far ahead about future aspects and to work in a detailed/analytical way, this was especially difficult when the project was not concrete yet and no Italian project examples were available. They experienced these difficulties amongst others, when filling in the *checklist on expected facilitators and barriers*. As explained by one of the stakeholders:

"The first meetings were a bit difficult, because we had to do all the discussions of the checklist about barriers and facilitators. For us Italians, at least that was absolutely the most difficult thing". (R1)

They found it very time consuming to fill in the checklist. Even though, they considered the general principal of the checklist as useful. It helped some members to get new insights and to start the preparatory work more informed.

Macro level
The stakeholders considered obtaining sufficient financial resources to be a crucial aspect in order to develop the project. At the start they expected difficulties in obtaining financing, but they could not think of possible solutions to solve this problem. Obtaining financing was indeed experienced to be difficult, they explained that their possibilities were limited, due to lack of time and the Italian financial framework for health care. One stakeholder described, that in order to obtain *financing by sanitary services,* much time was needed to first make an assessment of the activities organized in the centres. In addition, there was not enough time to apply for *state or regional financing.* As explained by one of the members of subgroup III (financing and location):

"To finance these, new projects, it is a long path. Because you can have financing from the region or the state, but it takes years to obtain this financing". (R11)

Eventually, they found two funding organizations who each financed one centre.
The stakeholders expected that one person with *knowledge about health insurance regulations and norms* would be more useful than having knowledge scattered amongst members. They were able to involve one person with this knowledge in the project, which saved time in studying *laws and regulations*. Because the location was already adapted to elderly persons, laws and regulations regarding the location were also less of a concern for the IG.

DISCUSSION

The purpose of this study was to investigate the preparation of the adaptive implementation of the proven effective Dutch Meeting Centres Support Program for people with dementia and their carers (MCSP) in Milan. The main focus in our study was to investigate factors that were experienced as facilitating or impeding the (preparation phase of the) implementation of this support programme. The results of this study show that, several preconditions and factors specific for the preparation phase impeded and/or facilitated (the preparation phase of the) implementation. Some of these factors were expected to be facilitating and/or impeding at the start of the implementation project, while others were not foreseen. One important unexpected facilitating precondition which made it easier to obtain the necessary resources, was the use of *existing networks and collaborations.* A major impeding unforeseen precondition was *lack of time* for the preparation phase because the actual opening had to be speeded up with four months because of financing opportunities. An already expected facilitating factor, specific for the preparation phase, was the *motivation of IG* members*.* Unexpected impeding factors specific for the preparation phase were: poor *collaboration with other users of the selected location for one of the meeting centres,* and not being used to aspects of the general *working method in the project* (working according to a detailed stepped plan) used for the preparation of the implementation.

FINDINGS IN THE LIGHT OF LITERATURE

Many different factors were experienced to impede or facilitate the MCSP implementation. This is in agreement with the idea of Grol [28] who described that many factors at social, organizational, financial and professional level can affect implementation, and these cannot be portrayed in one single strategy. During implementation unexpected events can occur even when a solid project plan is available, due to environmental impacts which change the plan [29]. This was also experienced in the current study, and therefore some impeding and facilitating factors were not expected by the stakeholders at

the start. For example, lack of time was not expected at the start of the project because there was enough time scheduled for the preparation phase. Other facilitating and impeding factors may not have been foreseen because many stakeholders indicated that they found it difficult to imagine how things would go in practice and found it difficult to think about future potential facilitating or impeding factors.

One of the major success factors for the implementation of this project was the use of existing networks and collaborations. A qualitative study by van Haeften-van Dijk et al. [22] about the transformation of day care centres in nursing homes into community day care centres in the Netherlands, also showed that stakeholders with an existing collaborative network made it easier to create collaborations for the purpose of the implementation of the new community day care centres and united (in principal) different interests of organizations. In our study, for example, it improved the difficult collaboration with other users of the location. In addition, it was crucial in order to obtain the necessary resources and to make the project practically feasible.

Collaboration between stakeholders is a rather complex process, in which different agendas, cultures and priorities can play a role, as shown in a qualitative implementation study of Aarons et al. [30]. Also in the current study the collaboration with the other users in one location was found to be difficult, as they had different priorities and views on the project. Due to the complexity of this collaboration, it is expected to remain a delicate topic also in the next phases of implementation: the execution and continuation phase. Furthermore, the collaboration of IG members could have been quite difficult, due to different backgrounds and interests. However, the communal motivation united the members to collaborate as a group.

Shortage of time impeded several preparatory tasks of the current study. The Dutch MCSP implementation study of Meiland et al. [23] emphasizes the importance of taking enough time to prepare the implementation of the meeting centres. For example, to gain support by organizations in the region and to spread information to referral organizations for the recruitment of participants. However, it was no option to take more time for the preparation in the Milan project. As a consequence, several tasks of the preparation phase had to be postponed, which is likely to cause a higher workload in the execution and continuation phase.

A systematic review of Gearing et al. [31] on cross-cultural adaptation and translation of mental health interventions, shows that there can be many difficulties regarding the cultural adaptation and translation of interventions. According to the cross cultural communication model of Lewis [32], the Italian culture is a complete multi-active culture which means that, they only plan the grand outlines and they are flexible in their agenda. In contrast, the Dutch culture is almost a complete linear active culture which means that, they plan ahead step by step and stick to the agenda. Originally, the plan was to have an adaptive implementation of the Dutch MCSP as it was foreseen that for every new implementation it is important to address needs, preferences and characteristics of each local situation. Yet, some Italian stakeholders were not used to aspects of the working method for adaptive implementation, and therefore still experienced difficulties in carrying out the preparatory tasks. This might indicate that the working method needs further adaptation allowing to adopt a methodology that fits to the specific culture, and helps stakeholders to think about detailed, non concrete, and future aspects of the project.

STRENGTHS AND LIMITATIONS

The results of this study were partly obtained by interviewing the majority of the stakeholders (n = 13) involved in the preparation phase. These interviews were double coded by two independent researchers (MS and RC) in order to improve reliability. In addition, the theoretical model of Meiland et al. [24] was used as a guide to trace facilitators and barriers. The validity is increased by sending a summary of the results to the stakeholders and by requesting their comments. However, the interviewed stakeholders also had to evaluate their own work and role in the project which might have influenced their objectivity. Furthermore, the impeding and facilitating factors are not all experienced in the same way by all stakeholders. This may be due to differences in roles and interests in the project or because of a lack of communication. To gain a comprehensive understanding of the different views it would have been interesting to additionally perform a focus group.

IMPLICATIONS FOR RESEARCH AND PRACTICE

The MCSP is in line with the first Italian national plan of dementia (approved in 2014), since it has an integrated approach, it increases the knowledge about dementia and it may improve the quality of life [33]. The implementation of MCSP was investigated for the Dutch situation [23], however no implementation study has been performed yet in the Italian context. Moreover, the results of this study on facilitating and impeding factors of implementation can be used for the further dissemination of MCSP in Italy and other European countries. For example, it is recommended to study the content of MCSP

in more detail at the start of the preparation phase, in order to get a better understanding of possible facilitators and barriers of the implementation of MCSP in their own region. In addition, cultural differences regarding the working method for adaptively implementing MCSP need more attention, for example giving more explanation and/or guidance to some existing tasks in the project or adapting the current tasks more to the way of working of the stakeholders (i.e. more concrete examples to help imagine possible facilitators and barriers). Finally, it is crucial for future centres to take enough time to execute all preparatory tasks in an adequate manner, to ensure a successful implementation.

CONCLUSIONS

Overall it can be concluded that the Dutch MCSP is suitable for implementation in an Italian setting. The project can be facilitated even more if the working method for executing some of the different tasks would be further adapted to the Italian way of working. Furthermore, not all experienced facilitators and barriers were foreseen, such as the importance of having a network with also organizations who are not involved in dementia care. Many experienced facilitators and barriers were in line with the findings of the Dutch implementation research into MCSP. However, also new factors appeared to influence the implementation in Milan, such as the collaboration with other users of the location and cultural differences in working method. The knowledge on facilitators and barriers of implementation gained in this study, can be used for the further dissemination of MCSP in other regions of Italy and in other European countries.

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ORIGINAL INVESTIGATION

Mood disorders in elderly patients hospitalized for acute exacerbation of COPD

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Chronic obstructive pulmonary disease (COPD) represents the most common cause of chronic respiratory failure and it is associated with several comorbidities such as depression.
Depression is about four times more frequent in elderly patients with COPD compared to peers who are not affected and its prevalence increases with the degree of disease severity.
The aim of our study was to assess mood and perception of the quality of life in elderly patients hospitalized for acute exacerbation of COPD. For this purpose 35 elderly patients (20 M and 15 F; average age 75.2 ± 6.4 years) hospitalized for reactivation of COPD were examined; they were subjected to spirometry test for the calculation of FEV1 and to CAT and HAM-D.
Findings show that a greater severity of depressive symptoms is related to a greater severity of COPD exacerbations, disability associated with it and perceived by the patient, as well as a higher number of recovery days and annual acute exacerbations, in particular in female gender.

Key words: Depression, Elderly, COPD

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) represents the most common cause of chronic respiratory failure and it is associated with increasing disability, morbidity and mortality. In the United States, in fact, it is the fourth leading cause of death and the fifth cause of disability and according to World Health Organization (WHO) it will be the third leading cause of death by 2030, second only to cardiovascular diseases and cancer [1]. In Italy an estimate of the prevalence confirms that COPD affects a significant portion of the adult population, about 4.5%, with a gradual age-related increase up to 20% in the population aged 65 years or over [2 3].
Depression is about four times more frequent in elderly patients with COPD compared to peers who are not affected and its prevalence increases with the degree of disease severity.
The estimated prevalence of depression in patients with stable COPD ranges between 10% and 42%, between 19.5% and 50% in patients with exacerbations of COPD and up to 60% in patients on long-term oxygen therapy [4 5].

MATERIALS AND METHODS

The aim of our study was to assess mood and perception of the quality of life in elderly patients hospitalized for acute exacerbation of COPD, as several studies have shown that depression in adult patients is associated with a worse prognosis in terms of quality of life and life expectancy. It is also a predictor of hospital length of stay (LOS), hospital readmissions and use of health care resources.

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For this purpose 35 elderly patients (20 M and 15 F; average age 75.2 ± 6.4 years) hospitalized for reactivation of COPD were examined.
The severity of exacerbations was assessed, during the first day of hospitalization, by spirometry [6]; this test was performed to calculate the forced expiratory volume in one second (FEV_1) expressed as a percentage of predicted values for the patients of similar characteristics (sex, age and height).
COPD Assessment Test (CAT) [7] and Hamilton Rating Scale for Depression (HAM-D) [8] were used to evaluate impact of COPD on the patient's quality of life and depressive symptomatology, respectively. The number of COPD exacerbations, defined according to Anthonisen criteria [9], shown by each patient in the last year prior to hospitalization, was also recorded.
Patients with severe comorbidities according to the score of Cumulative Illness Rating Scale (CIRS > 3), on long-term home oxygen therapy (OLT) and treated with antidepressant drugs were previously excluded.
At the end of the hospitalization we have calculated the number of recovery days required for the stabilization of patients and the discharge home.
Statistical analysis of the data was done using software IBM SPSS- version-21.0.

RESULTS

Sample characteristics are shown in Table I. According to CAT test, 20% of patients (6 M and 1 F) had low CAT score, 45.7% (9 M and 7 F) had average CAT score, 14.3% (2 M and 3 F) had high CAT score and 20% (3 M and 4 F) had very high CAT score.
Concerning the mood, the results of HAM-D test showed that 13 patients (9 M and 4 F) were not depressed (score < 8), 13 patients (8 M and 5 F) had mild depression (score 8-16), 9 patients (3 M and 6 F) had moderate depression (score 17-23) and no one had severe depression (score > 23). Globally 73% of female partecipants had depression as compared to 55% of males.
The spirometric evaluation of FEV1 showed a mean percentage score of 51 ± 11.8, 55 ± 11.3 *versus* 47.3 ± 11.3 in males and females, respectively.
The mean number of exacerbations in the last year was 1.6 ± 1.4, with mean values of 25.1 ± 1.3 in men and 1.9 ± 1.5 in women.
The registered average length of stay was 8.6 ± 3.2 days, longer in females (9.8 ± 3) than males (7.7 ± 3).
Sperman and Mann-Whitney's correlations were employed to determine the relationship between the examined variables.
There were strongly significative correlations ($p < 0.001$), positive between HAM-D scores, CAT scores (Fig. 1), number of exacerbation in the last year and hospital length of stay (Fig. 2), and negative between HAM-D scores and FEV1 values (Fig. 3). Furthermore, females were more depressed, with lower FEV1 ($p = 0.043$) and with a longer length of stay ($p = 0.039$) as compared to males.

Table I. Characteristics of the sample.

Characteristics	Males		Females		Total	
	N (%)	Mean ± SD	N (%)	Mean ± SD	N (%)	Mean ± SD
Sex	20 (57.1)	-	15 (42.9)	-	35 (100)	-
Age	-	74.4 ± 5.67	-	76.3 ± 7.44	-	75.3 ± 6.45
Weight	-	73.1 ± 9.8	-	67.7 ± 15.8	-	70.7 ± 12.87
Height	-	165.3 ± 1.43	-	157.1±1.53	-	161.8±7.37
FEV1	-	55 ± 11.3	-	47.3 ± 11.3	-	51.74 ± 11.84
CAT	-	15 ± 9.11	-	20.8 ± 9.48	-	17.5 ± 9.59
Low score	6	-	1	-	7 (20)	-
Average score	9	-	7	-	16 (45.7)	-
High score	2	-	3	-	5 (14.3)	-
Very high score	3	-	4	-	7 (20)	-
HAM-D	-	8.95 ± 5.2	-	12.73 ± 5.57	-	10.6 ± 5.61
Depression	11	-	11	-	22 (62.9)	-
- Mild	8	-	5	-	13 (37.1)	-
- Moderate	3	-	6	-	9 (25.8)	-
- Severe	0	-	0	-	0	-
No depression	9	-	4	-	13 (37.1)	-
Lenght of stay (LOS)	-	7.7 ± 3	-	9.8 ± 3	-	8.6 ± 3.2
Exacerbations in the last year	-	1.25 ± 1.3	-	1.9 ± 1.5	-	1.6 ± 1.4

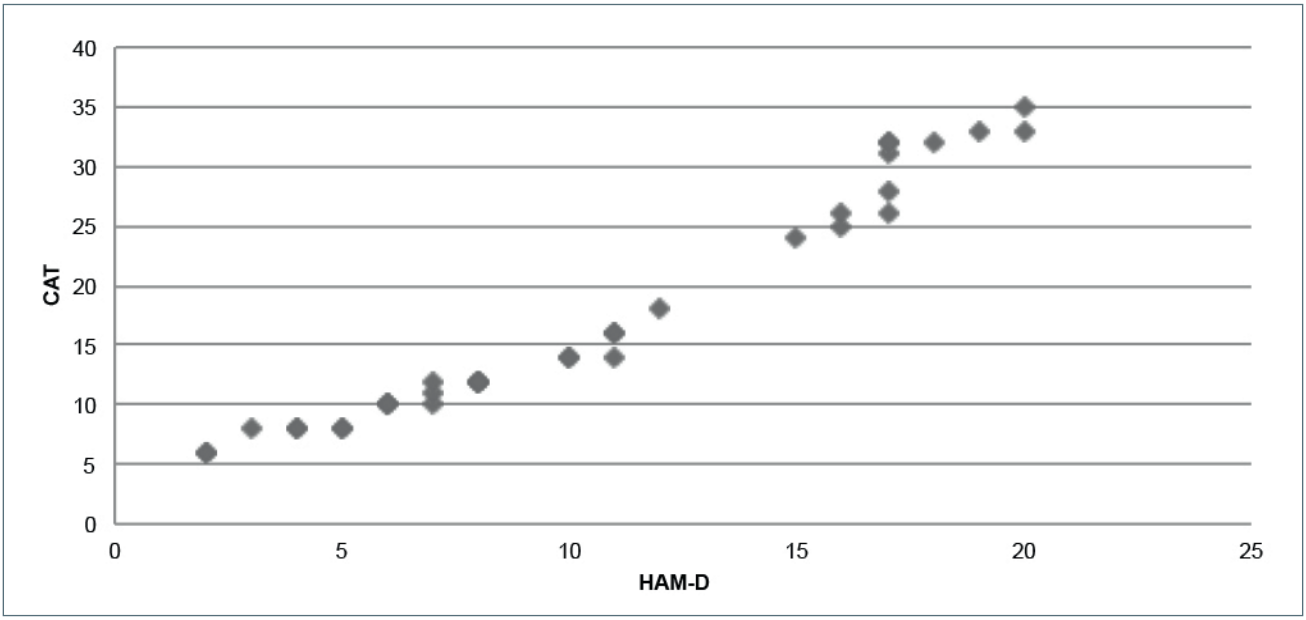

Figure 1. Correlation between CAT and HAM-D scores.

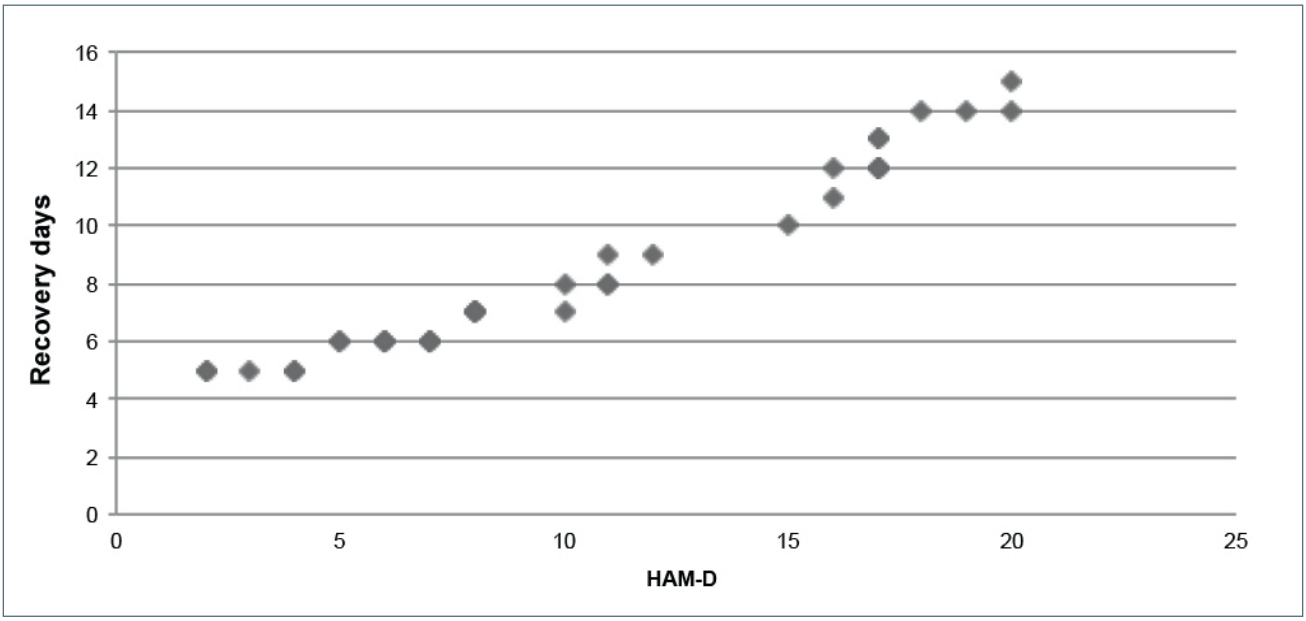

Figure 2. Correlation between hospital lenght of stay and HAM-D scores.

DISCUSSION

Chronic obstructive pulmonary disease (COPD) is a pathological respiratory condition characterized by airflow obstruction, to which alteration of bronchi (chronic bronchitis), bronchioles (disease of the small airways) and lung parenchyma (emphysema) contribute, induced by inhaling harmful substances (especially tobacco smoke) which determine a chronic inflammatory state [1 11].

The diagnosis of COPD is based on the presence of respiratory symptoms (cough, chronic sputum production, dyspnea), on a history of exposure to risk factors and on the evidence of airway obstruction using spirometry [2 6].

Although COPD is primarily recognized as a respiratory disease, it is not limited to the respiratory system but spreads to a systemic level inducing further organ damage. In fact, in addition to causing COPD, smoking, the

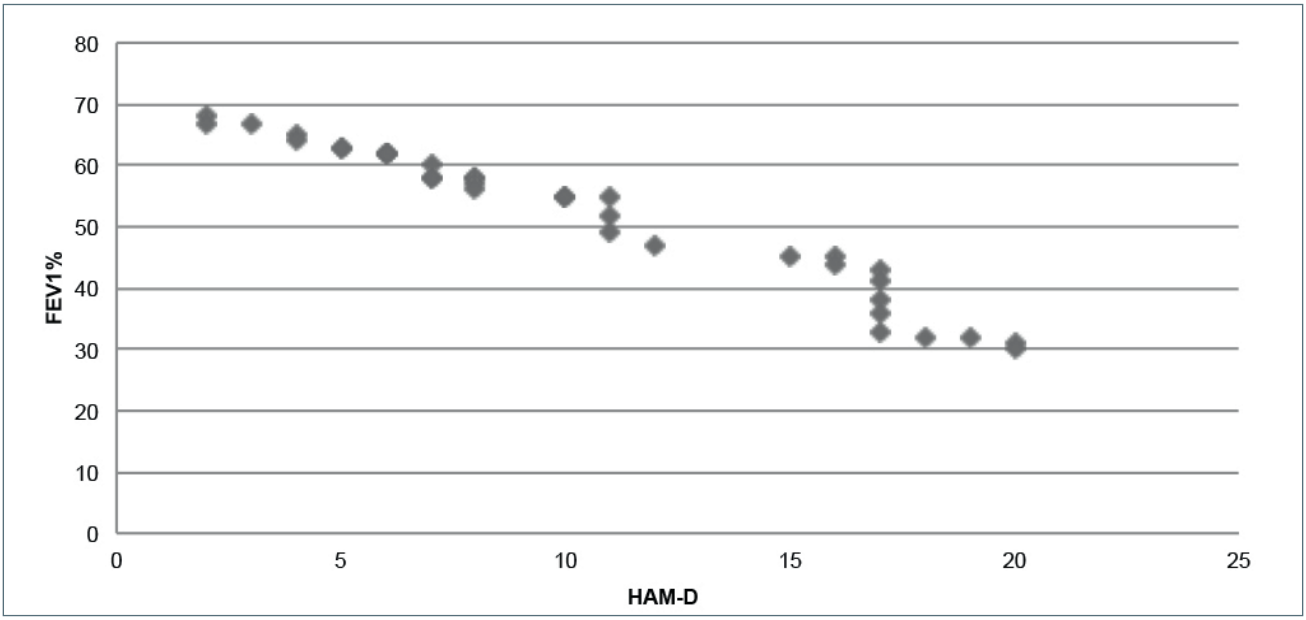

Figure 3. Correlation between FEV_1 values and HAM-D scores.

first cause of COPD, has systemic effects which can contribute to the development of chronic diseases including cardiovascular, metabolic, kidney diseases and tumors, along with other risk factors such as hyperlipidemia, obesity, hypertension and sedentary lifestyle [4]. COPD and chronic diseases associated particularly develop in the elderly, and aging itself constitutes an amplifying factor for their development in synergy with the risk factors mentioned above [7]. Psychiatric comorbidities should certainly be counted among the systemic manifestations of COPD. Depression in particular is seen in COPD more often than not, worsening the patients' level of disability and their perception of the quality of life [5 6 12].

In our study, in fact, using HAM-D as screening test, we found that a large proportion of the enrolled population (about 63%) had depressive symptoms, especially women (about 73%).

Regarding the impact of COPD on quality of life assessed by CAT questionnaire, it was found a high degree of disability in 35% of patients, while only 20% did not report debilitating symptoms due to respiratory disease.

Our sample of hospitalized elderly patients also had a reduction in FEV1, compared to the predicted normal value, around 50% in agreement with the international studies in which a decline of FEV_1 less than 50% of its theoretical value is related to a sharp deterioration of health status and rate of hospitalization [13-15].

The days of hospitalization necessary for clinical stabilization and discharge home were higher in females (about 10 days) than the average (about 8 days) and males (about 7 days). In addition, women had a number of exacerbations about twice the average of the sample.

As it was assumed based on international studies on adults [16-18], in the current study on geriatric inpatients, depression and COPD-related disability have emerged as an important problem among elderly. In fact we found highly significant correlations ($p < 0.001$), positive between HAM-D scores, CAT scores, number of exacerbation in the last year and hospital length of stay, and negative between HAM-D scores and FEV1 values, such that a greater severity of depressive symptoms is related to a greater severity of COPD exacerbations, disability associated and perceived by the patient, as well as a higher number of recovery days and annual acute exacerbations.

Just as it is shown in the adult population [19-21], older women hospitalized for COPD had a greater impairment of mood with depressed mood, a worse perception of their quality of life, a number of exacerbations about twice the average of the sample and a longer hospitalization. Thus, HAM-D test is an excellent indicator of depression in the elderly and to represent the impact of COPD on mood and consequently on the perception of the quality of life, just like CAT questionnaire, especially in female people. If it were administered at the time of admission to hospital, as well as spirometry and CAT, it could be used for predicting the hospital length of stay and even the rehospitalization rate, probably because most depressed patients are less adherent to long-term

pharmacoterapy that appears necessary to reduce the frequency of exacerbations, hospitalization and so the health expenditure. Further studies are needed to assess the impact of antidepressant treatment on adherence to drug therapy and frequency of exacerbations in older adults with COPD, especially females, identified by HAM-D.

CONCLUSIONS

Depression is an important comorbidity in elderly hospitalized patients with COPD, especially in women, as it is related to a worse prognosis in terms of both quality of life and life expectancy. Besides, it is emerging as a good predictor of rehospitalization and length of stay. In this regard, the early diagnosis of affective disorder in elderly patients with COPD, followed by administration of HAM-D test, could be a valuable tool for improving the quality of patients' lives, adherence to treatment and so reducing early rehospedalizations in order to allow a less expenditure of health care resources.

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JGG 2017;65:18-37

ORIGINAL INVESTIGATION

Routine invasive mediastinal staging of lung cancer in elderly patients without lymph adenopathy on PET-CT scan: is an appropriate choice?

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We have reviewed the literature to clarify if routine invasive mediastinal staging is indicated also in Stage I elderly patients screened with PET/CT scan. Nineteen papers were chosen to answer the question. Occult pN2 disease was < 10% in five papers; between 10-16% in four papers; and > 16% in four papers.Significant risk factors for occult pN2 disease are the SUV value of primary tumor (seven papers), central tumor (four papers), tumor > 3 cm (five papers), adenocarcinoma histology (five papers) and cN1 disease(two papers). Two papers found that unexpected pN2 patients had a better survival than cN2 patients operated after induction therapy. Invasive mediastinal staging is recommended also in cN0 patients with central tumor or with peripheral tumor > 3 cm.

Key words: CT/PET, Invasive technique, Mediastinal staging, Non small cell lung cancer

INTRODUCTION

Increases in both life expectancy and cancer incidence with age, together to the exposure to pollutants including smoking habit, result in a significant rise in lung cancer rates among elderly patients [1-5].
At diagnosis, half of the patients are over 70 years of age, and most present with comorbidities and advanced disease for which chemotherapy provides limited benefit in terms of response rate and survival [6-16].
Better understanding cancer biology [17-37] is leading to renovated target based approaches also in elderly. Mediastinal lymph node (LN) staging represents the cornerstone in the diagnosis, treatment and prognosis of patient with non-small cell lung cancer (NSCLC). Despite the advances in radiological proceduresand the routine use of F-18 fluorodeoxy-D-glucose positron emission tomography(18-FDG-PET) [38-45] in diagnostic work-up of lung cancer, 5-15% of NSCLC patients clinically staged as N0 and undergoing surgery have an unexpected pN2 disease [46-54].

Thus, we have reviewed the literature to define if routine invasive mediastinal stage is indicated in NSCLC elderly patients without LN involvement on PET-CT, an issue still debate.

RESEARCH CRITERIA

Medline search was done on PubMed, EMBASE and Cochrane databases using the following terms: lung cancer, mediastinum, PET, staging, Endoscopy (Bronchial) Ultrasound-Endoscopy (EBUS, EUS), Video Assisted Thoracic Surgery (VATS), and mediastinoscopy. The time frame was restricted to articles published from January 2000 up to July 2015. Cited references of review articles on indication for invasive mediastinal staging were manually examined to find additional articles not found in the computerized databases. Additional articles were identified from reference lists of selected articles. No-English language papers, case reports, abstracts only, letters and unpublished data were excluded. Of the 293 papers founded, 19were identified for answering our question and summarized in Table I.

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RESULTS

Park et al. [55] attended mediastinoscopy in 78/147 (53%) patients with NSCLC Stage I. N2 disease was found in 7 (4.8%) cases of which 6 underwent mediastinoscopy with diagnosis of N2 involvement in only 3 cases (50%). Significant predictors of N1/N2 metastasis was a SUV of primary tumor > 7.3 (p = 0.001).

Cerfolio et al. [56] evaluated 153 NSCLC cN0 (n = 136) and cN1 (n = 17) patients screened with PET/CT. All patients underwent mediastinoscopy and EUS. N2 disease was found in 22/153 (14.3%) patients; among cN0 (n = 15) mediastinoscopy (n = 4; 2.9%) and EUS (5; 3.7%) correctly diagnosed N2 disease in 9 cases and failed in 6 (4.7%); among cN1 (n = 7) mediastinoscopy (n = 3; 17.6%) and EUS (n = 4; 23.5%) correctly diagnosed N2 disease in all cases. Significant risk factors were a SUV primary tumor > 10 (0.01) and poorly differentiated cancer (0.03).

Sivrikoz et al. [57] attended mediastinoscopy in 68 resectable patients. N2 disease was found in 11/68 (16%) cases. Mediastinoscopy correctly diagnosed N2 diseases in 9/11 patients (81.8%) and failed in 2/11 because sub-centimeters LNs.

Sanli et al. [58] studied 78 NSCLC patients. Mediastinoscopy (n = 33/78; 42%) was attended in cN2 patients and in those with adenocarcinoma or central tumors even without mediastinal involvement. Accuracy of mediastinoscopy was 96.9% with one false negative result.

Al-Sarraf et al. [59] evaluated 153 NSCLC patients without mediastinal adenopathy. No mediastinoscopy was performed. N2 disease was found in 25/153 (16%) patients; significant risk factors were central tumor (p = 0.007); right upper lobe (p = 0.01); and cN1 disease on PET (p = 0.002).

Perigaud et al. [60] evaluated 51 NSCLC. Mediastinoscopy was attended in only 2 patients to exclude N3 disease. N2 disease was found in 10/51 (19.6%) patients; of these, 6 sub-centimeters LNs were PET negative. Meyers et al. [61] evaluated 248 NSCLC early-stage patients. 14/248 (5.6%) had N2 disease; of these 13/14 (92.8%) underwent mediastinoscopy detecting metastasis in 5/13 (38%) patients. Only 1/70 patient who did not have mediastinoscopy had N2 disease. The 5 year progression free survival of patients undergoing mediastinoscopy or not was similar (72% *vs* 77%; p = 0.245). Zhang et al. [62] in 530 NSCLC T1N0 stage patients found N2 disease in 89/530 (16.8%) cases. No mediastinoscopy neither PET/CT was routinely carried-out. Significant risk factors were central tumor (p = 0.002); tumor size (p < 0.001), and invasive adenocarcinoma (p < 0.001). De Franchi et al. [63] in 968 pT1 patients found 59/968 (6.1%) occult N2 diseases. In 16/59 cases (27%) mediastinoscopy was attended revealing N2 disease in 3/16 (19%) cases and failing in 13 (81%). In 7/13 cases, metastases were in stations not accessible by mediastinoscopy whereas in 6/13 cases in 4R or 7 stations. The 5 year-survival-time of patients with occult N2 disease was better than cN2 patients (46% *vs* 31%).

Lee et al. [64] attended mediastinoscopy in 76/224 (34%) NSCLC Stage I patients. N2 disease was found in 16/224 (7.1%). Metastases were identified by mediastinoscopy in 11 and missed in 5 cases. Significant risk factors were central tumor location (p < 0.001); tumor size > 6.0 (p < 0.001), and SUV > 4.0 (p = 0.01). Kim et al. [65] found occult N2 disease in 34/150 (23%) cases. PET-CT had a low value of sensitity (47%) probably because LNs were sub-centimeters. Thus, the authors concluded that negative PET N2 disease did not obviate mediastinoscopy. Iskender et al. [66] evaluated 212 patients with NSCLC underwent to PET/CT and mediastinoscopy. Only 4/107 (3.7%) with negative mediastinal LN uptake on PET/CT had pN2 disease. Trister et colleagues [67] drew up a report, focusing on 201 patients with clinical stage I and II NSCLC screened with PET scan and undergoing invasive staging of the mediastinum. N2 disease was found in 63/201 (31%) patients. Multivariate analysis showed thatSUV of primary tumour > 6 was the only significant predictive factor (p = 0.02). Gomez-Caro et al. [68] investigated 79 patients with NSCLC Stage I screened with PET-CT scan. Occult pN2 diseases were found in 6/79 (7.6%) among patients with Stage IA and 11/74 (14.8%) among those with clinical Stage IB. Significant risk factors for occult pN2were tumor sizes ≥ 5 cm, pN1 disease, adenocarcinoma and female patients. Wang et al. [69] in a metanalysis including 10 studies and a total of 1122 patients with NSCLC stage I (T1-2N0) NSCLC evaluated the negative predictive value of PET-CT. Negative predictive value of PET/CT in detecting of mediastinal LN metastases was 94% in T1 and 89% in T2 patients. Significant risk factors were adenocarcinoma histology and high FDG uptake of the primary lesion.

Billé et al. [70] investigating 353 NSCLC stage I patients. PET/CT sensitivity, specificity and accuracy were 38.8%, 97.4%, and 85.7% for adenocarcinoma histology and 81.8%, 91.8% and 90.8% for squamous carcinoma histology.

The authors [71] evaluated 901 consecutive patients with Stage I NSCLC screened with PET/CT scan. 108/901 (12%) had unexpected pN2 disease. Central tumor location (p < 0.003), cT2a (p < 0.0001) and pT2a stage (p < 0.0001), pN1 disease (p = 0.004), and SUV of primary tumor > 4.0 (p = 0.007) were prognostic factors of occult pN2 disease. pN2 patients versus cN2 patients operated after induction therapy presented a betteroverall survival (56 *vs* 20 months; p = 0.001) and disease-free survival (46 *vs* 11 months; p < 0.0001).

<table>
<tr><th>Author, date and country, Study type (level of Evidence</th><th>Patient group</th><th>Outcomes</th><th>Key results</th><th>Comments</th></tr>
<tr><td>Park et al (2010). Respirology Korea [8]

Retrospective Single centre case series</td><td>From January 2005 to December 2007, 147 patients diagnosed as clinical stage IA by integrated PET-CT were enrolled

78/147 (53%) had preoperative mediastinoscopy
144/147 (98%) underwent resection with radical lymph adenectomy
3 patients were excluded from surgery because N2 involvement</td><td>N1 disease
N2 disease

Accuracy of mediastinoscopy

Predictors of N1/N2 disease</td><td>9.5% (14/147)
4.8% (7/147)

<table><tr><th>Occult N2 disease</th><th>Mediastinoscopy yes</th><th>Positive Mediastinoscopy</th><th>Negative Mediastinoscopy</th></tr><tr><td>4.8% (7/147)</td><td>6/7 (85%)</td><td>3 (50%)</td><td>*3 (50%)</td></tr></table>* Metastasis was missed because of false negative (n=1) or inaccessible nodal groups (n=2)

<table><tr><th>Characteristics</th><th>Odds Ratio</th><th>p</th></tr><tr><td>Age, years</td><td>1.860</td><td>0.237</td></tr><tr><td>Gender</td><td>0.985</td><td>0.594</td></tr><tr><td>SUV max primary tumor >7.3</td><td>7.574</td><td>0.001</td></tr><tr><td>Tumor size, cm</td><td>1.233</td><td>0.721</td></tr></table></td><td>The higher SUV max > 7.3 was an independent predictor of occult nodal metastasis in patients with clinical stage IA NSCLC. Because routine mediastinoscopy was not performed in all patients, its role in such patients remained unclear.</td></tr>
</table>

Right lower lobe tumor	47 (36%)	10 (45%)	0.4
Median tumor size	1.8	2.3	0.05

<table>
<tr>
<td>Sivrikoz et al (2012); Thorac Cardiovasc Surg Turkey [10]

Prospective Single centre case series</td>
<td>From February 2007 to May 2010, 68 with resectable NSCLC and undergoing integrated PET/CT were evaluated

All patients underwent standard and extended cervical mediastinoscopy and resection with radical lymph adenectomy if mediastinoscopy was negative</td>
<td>N2 disease

Diagnostic accuracy</td>
<td>16% (11/68)

<table>
<tr><th>Methods</th><th>Sensitivity (%)</th><th>Specificity (%)</th><th>PPV (%)</th><th>NPV (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Mediastinoscopy in all pts</td><td>81.8*</td><td>100</td><td>100</td><td>96.6</td><td>97</td></tr>
<tr><td>PET/CT in all pts</td><td>61**</td><td>98</td><td>91.7</td><td>87.5</td><td>88.2</td></tr>
<tr><td>PET/CT in only N2 and N3 pts</td><td>72.7</td><td>97.7</td><td>88.9</td><td>93.3</td><td>92.6</td></tr>
</table>
*In 2 cases, occult N2 disease was missed because lymph node < 1 cm

**3 patients with occult N2 disease (lymph node < 1cm) and 4 patients with N1 occult diasease (central tumor) were false negative</td>
<td>Positive PET results must be pathologically confirmed. Routine mediastinoscopy can be omitted in patients with negative PET/CT for mediastinal lymph node</td>
</tr>
<tr>
<td>Sanli et al (2009) Journal of Thoracic and Cardiovasc Surg, Turkey</td>
<td>From March 2006 to June 2008, 78 patients with NSCLC were enrolled.</td>
<td>Diagnostic accuracy of PET-CT scan</td>
<td>
<table>
<tr><th>Node station</th><th>Sensitivity (%)</th><th>Specificity (%)</th><th>PPV (%)</th><th>NPV (%)</th><th>Accuracy (%)</th></tr>
<tr><td>N2</td><td>81.8</td><td>89.5</td><td>56.2</td><td>96.7</td><td>-</td></tr>
<tr><td>N1</td><td>34.6</td><td>88.8</td><td>64.2</td><td>70.1</td><td>69</td></tr>
</table>
</td>
<td>Mediastinoscopy is required in patients with positive</td>
</tr>
</table>

<table>
<tr>
<td>[11]

Prospective Single centre case series</td>
<td>All patients were clinically staged using integrated PET-CT scan.

Mediastinoscopy was attended in N2 clinically patients and in patients with a histology of adenocarcinoma or having central tumors even if N2 was not detected in radiological examinations.</td>
<td>Diagnostic accuracy of PET-CT scan compared to surgical stage

Accuracy of mediastinoscopy</td>
<td>
<table>
<tr><th>Up-stage</th><th>Down stage</th><th>Univariate</th></tr>
<tr><td>16</td><td>12</td><td>50</td></tr>
</table>
<table>
<tr><th>Number of procedures</th><th>True negative</th><th>False negative</th><th>True positive</th><th>Accuracy (%)</th></tr>
<tr><td>33/78 (42%)</td><td>25</td><td>1</td><td>7</td><td>96.9</td></tr>
</table>
</td>
<td>mediastinal lymph node on PET-CT scan but it might not be necessary in patients without radiological lymph nodes involvement</td>
</tr>
<tr>
<td>Al-Sarraf et al (2008), Eur. J. Cardio-Thorac Surgery, Ireland [12]

Retrospective Single centre case series</td>
<td>Over 30 period months, 153 patients with NSCLC undergoing curative intentent surgical resection were</td>
<td>N2 disease</td>
<td>25/153 (16%) patients
16/25 (64%) within station 7
7/25 (28%) within station 4</td>
<td>Patients with centrally located tumor, with right upper lobe tumors, and with positive N1 lymph node on PET</td>
</tr>
</table>

<table>
<tr>
<td></td>
<td>enrolled.

All patients were staged using PET-CT scan which showed no uptake in the mediastinum. No preoperative mediastonoscopy was carryout.</td>
<td>N2 risk factors</td>
<td>
<table>
<tr><th>Variable</th><th>Odds Ratio</th><th>p</th></tr>
<tr><td>Central location</td><td>6.11</td><td>0.007</td></tr>
<tr><td>Right upper lobe</td><td>0.221</td><td>0.017</td></tr>
<tr><td>Positive N1 upatke on PET</td><td>0.164</td><td>0.002</td></tr>
</table>
</td>
<td>should have routine mediastinoscopy to rule out N2 metastasis especially in stations number 7 and 4.</td>
</tr>
<tr>
<td>Perigaud et al (2009), Eur. J. Cardio-Thorac Surgery, France [13]

Retrospective Single centre case series</td>
<td>From June 2006 to February 2008, 51 consecutive patients with NSCLC undegoing surgery. All patients were staged using integrated PET-CT scan. Peroperative mediastinoscopy was attended in</td>
<td>N2 disease

Diagnostic accuracy of integrated PET-CT</td>
<td>10/51 (19.6%) patients

<table>
<tr><th>Sensitivity</th><th>Specificity</th><th>PPV</th><th>NPV</th></tr>
<tr><td>40 ±30*</td><td>85 ±11</td><td>40 ±30</td><td>85 ±11</td></tr>
</table>
*In 6 cases, the negative N2 PET results were due to sub-centimetres lesions.
</td>
<td>Positive mediastinal lymph node on integrated PET-CT scan required invasive procedure as mediastinoscopy to exclude fasle positive results. In contrast,</td>
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<table>
<tr>
<td></td>
<td>only 2 patient to exclude N3 disease.</td>
<td></td>
<td></td>
<td>patients without mediastinal involvement on integrated PET-CT scan, can be operated without invasive procedures.</td>
</tr>
<tr>
<td>Meyers et al. (2006).
J. Thorac and Cardiov. Surg, USA [14]

Retrospective Single centre case series</td>
<td>From May 1999 to April 2004, 248 patients with clinical stage I of NSCLC were enrolled

All patients had preoperative integrated PET-CT

178/248 (72%) patients underwent preoperative mediastinoscopy</td>
<td>Occult N2 metastasis

Accuracy of mediastinoscopy

5 Year progression free survival (%)</td>
<td>14/248 (5.6%) patients

<table>
<tr><th>Occult N2 disease</th><th>Mediastinoscopy yes</th><th>Positive Mediastinoscopy</th><th>Negative Mediastinoscopy</th></tr>
<tr><td>14 (5.6%)</td><td>13/14 (92.8%)</td><td>5 (38%)</td><td>8 (61%)</td></tr>
</table>
Of 70 patients in whom mediastinoscopy was omitted , only a patient had N2 disease

<table>
<tr><th>All patients (n=229*)</th><th>Mediastinoscopy (yes) (n=169)</th><th>Mediastinoscopy (no) (n=60)</th><th>p</th></tr>
<tr><td>73</td><td>72</td><td>77</td><td>0.245</td></tr>
</table>
*6 patients with diagnosis of benign disease after resection were excluded</td>
<td>Routine mediastinoscopy in patients with clinically stage I lung cancer staged by PET and CT is clinically unproductive and excessively costly.</td>
</tr>
</table>

<table>
<tr>
<td>Zhang et al (2012)
J Thorac and Cardiovasc Surg . China
[15]

Retrospective Single centre case series</td>
<td>From June 2007 to August 2011, 530 patients with NSCLC clinically staged as T1N0 and undergoing surgical resection with radical lymphadenectomy were enrolled.

PET scan was not routinley used in clinical stage</td>
<td>N2 disease

N2 risk factors</td>
<td>89/530 (16.8%) patients

<table>
<tr><th>Variable</th><th>OR</th><th>95%CI</th><th>p</th></tr>
<tr><td>Age</td><td>0.974</td><td>0.952-0.997</td><td>0.025</td></tr>
<tr><td>Tumor Size (cm)</td><td>2.769</td><td>1.818-4.217</td><td><.001</td></tr>
<tr><td>Central location</td><td>3.204</td><td>1.512-6.790</td><td>0.002</td></tr>
<tr><td>Invasive adenocarcinoma</td><td>3.537</td><td>1.740-7.191</td><td><.001</td></tr>
</table>
</td>
<td>No use of routine mediastinoscopy in patients with NSCLC clinically staged as T1N0</td>
</tr>
<tr>
<td>Defranchi et al (2009)
Ann Thorac Surg USA
[16]

Retrospective Single centre case series</td>
<td>Between 1998 and 2006, 968 patients with pT1 NSCLC undergoing surgical resection were</td>
<td>N2 disease

Diagnostic accuracy of mediastinoscopy</td>
<td>59/968 (6.1%) patients

<table>
<tr><th>N2 disease</th><th>Mediastinoscopy yes</th><th>Positive Mediastinoscopy</th><th>Negative Mediastinoscopy</th></tr>
<tr><td>59 (6.1%)</td><td>16 (27%)</td><td>3(19%)</td><td>*13 (81%)</td></tr>
</table>
*In 7 cases, lymph node metastasis were found in stations not accessible by standard mediastinoscopy (stations 9 ;5; and 6)</td>
<td>For patients with T1 NSCLC and negative mediastinal imaging, routine mediastinoscopy results</td>
</tr>
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<tr>
<td></td>
<td>enrolled.

CT scan was perfromed in all patients while PET in 27 (46%) of cases.

Mediastinoscopy performed in presence of significant lymph nodes observed on CT scan, by increased metabolic activity on PET or by surgeon preference</td>
<td>5YST</td>
<td>
<table>
<tr><th>All surgical T1N2 pts</th><th>Clinical N2 pts</th><th>Occult N2 pts</th><th>*p</th></tr>
<tr><td>41%</td><td>31%</td><td>46%</td><td>0.43</td></tr>
</table>
*Clinical N2 versus Occulr N2 pts
</td>
<td>in a low yield of occult N2 disease discovery</td>
</tr>
<tr>
<td>Lee et al (2007) Ann Thorac Surg USA [17]

Retrospective Single centre case series</td>
<td>From January 2000 to November 2006, 224 patients with clinical stage I NSCLC screened by CT and PET were enrolled Mediastinosc</td>
<td>N2 disease

Accuracy of mediastinoscopy</td>
<td>16/224 (7.1%)
<table>
<tr><th>Occult N2 disease</th><th>Mediastinoscopy yes</th><th>Positive Mediastinoscopy</th><th>Negative Mediastinoscopy</th></tr>
<tr><td>16 (7.1%)</td><td>16</td><td>11(19%)</td><td>*5 (81%)</td></tr>
</table>
*In only 1 case metastasis was in a station 5, not accessible by mediastinoscopy.
</td>
<td>Patients with centrally located tumors, large tumor size, hystology of adenocarcinoma, and a</td>
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<td></td>
<td>opy was attended in 76 (34%) cases</td>
<td>N2 risk fcators</td>
<td>
<table>
<tr><th>Variable</th><th>Occult N2 metastases (%)</th><th>p</th></tr>
<tr><td>Tumor location (central/peripheral)</td><td>21.6 vs 2.9</td><td><0.001</td></tr>
<tr><td>Tumor Size (cm) 0-2/2.1-4.0/4.1-6/>6.0</td><td>4.8/6.5/6.3/57.1*</td><td><0.001*</td></tr>
<tr><td>Histology (adeno/squamous carcinoma)</td><td>9.0 vs 0</td><td>0.082</td></tr>
<tr><td>SUV max (0-4.0/>4.0)</td><td>1.9/10.5</td><td>0.01</td></tr>
</table>
</td>
<td>PET uptake value > 4.0 should have mediastinoscopy to rule out N2 occult metastasis. In the other cases it is no indicated.</td>
</tr>
<tr>
<td>Kim et al (2006) Radiology Korea [18]

Prospective Single centre case series</td>
<td>From June 2003 to February 2005, 150 patients with resectable lung cancer in Stage I screened by PET and CT were enrolled

Mediastinoscopy alone (n=15), mediastinoscopy +</td>
<td>N2 disease

Accuracy of PET</td>
<td>34/150 (23%) per patients
55/568 (10%) per nodal stations

<table>
<tr><th>Variaable</th><th>Sensitivity (%)</th><th>Specificity (%)</th><th>PPV (%)</th><th>NPV (%)</th><th>Accuracy (%)</th></tr>
<tr><td>Per patients</td><td>47</td><td>100</td><td>100</td><td>87</td><td>88</td></tr>
<tr><td>Per nodal stations</td><td>42</td><td>100</td><td>100</td><td>94</td><td>94</td></tr>
</table>
</td>
<td>Considering the low value of sensitivity, negative PET results do not obviate mediastinoscopy for mediastinal nodal stage</td>
</tr>
</table>

<table>
<tr><td></td><td>thoractomy (n=101), and thoracotomy alone (n=34) were attended</td><td></td><td></td><td></td></tr>
<tr><td>Iskender et al (2012).
Acta chir belg [19]

Retrospective Single centre case series</td><td>212 patients diagnosed with NSCLC between September 2005 and March 2008 were evaluated by PET/CT. Standard cervical mediastinoscopy was performed in all patients, and simultaneous extended cervical mediastinoscopy was performed in 52 patients with left sided lesions</td><td>N2 disease

Diagnostic accuracy</td><td>N2 occult disease: 4/107 (3.7%)

<table><tr><th>Sensitivity PET/CT</th><th>Specificity PET/CT</th><th>PPV and PNV PET/CT</th><th>Accuracy PET/CT</th></tr><tr><td>93.8%</td><td>69.6%</td><td>57.1%</td><td>96.3%</td></tr></table></td><td>In patients with positive mediastinal lymph node uptake on PET/CT invasive mediastinal staging appears necessary for exact staging. Mediastinoscopy can be omitted in NSCLC patients with negative mediastinal uptake on PET/CT.</td></tr>
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Trister et al (2014). Am J Clin Oncol. [20] Retrospective single centre case series	201 patients with clinical stage I and II NSCLC screened with PET scan and unergoping invasive mediastinal staging	N2 disease	N2 occult disease: 63/201 (31%) Multivariate analysis showed that SUV of primary tumour > 6 was the only significant predictive fasctor (p=0.02) while histology, tumor location (central vs. peripheral), sex, and age were not predictive for occult N2 disease.	Pathologic staging of the mediastinum should be strongly considered in patients with high SUV of primary tumor also in presence of negative mediastinum on PET.
Gomez-Caro et al (2012). Eur J Cardiothorac Surg [21] Prospectly study	Between January 2007 and December 2010, 402 patients with potentially operable NSCLC enrolled. 153 surgically treated patients (79 cIA and 74 cIB cases) were prospectively	N2 disease Diagnostic accuracy	N2 occult disease founded: 6 of 79 patients (7.6%) in clinical stage IA 11 of 74 patients (14.8%) in clinical stage IB.	Principal risk factors to have occult (pN2) lymph nodes were tumour sizes ≥5 cm, pN1, adenocarcinoma and female patients. The report concluded

	included in the study. Non-invasive surgical staging was carried out in this group, and curative resection plus systematic mediastinal dissection was performed except in the event of unexpected oncological contraindi-cation.			that in tumours ≤1 cm (pT1a), surgical staging was unnecessary, while adenocarcinoma and non- central cIB required a more efficient invasive staging.
Wang et al Clin Lung Cancer. 2012 [22] Meta - analysis	Ten studies with a total of 1122 patients with stage I (T1-2N0) NSCLC analyzed from international literature	N2 disease Diagnostic accuracy	Negative predictive value of PET/CT in detecting of mediastinal lymph-node metastases is 94% in T1 and 89% in T2.	Risk factors of occult metastases are adenocarcinoma histology and high FDG uptake in the primary lesion. Low rate of NPV

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<td>suggested unnecessariness of routine invasive staging procedures for T1 subgroup of patients.</td>
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<td>Billé et al 2013 Eur J Cardiothorac Surg [23]

Retrospective study</td>
<td>353 consecutive patients with suspected or pathologicall y proven, potentially re-sectable non-small-cell lung cancer (NSCLC) who had integrated PET/CT scanning at the same centre. Lymph node staging was patho-logically confirmed on</td>
<td>N2 disease

Diagnostic accuracy</td>
<td>PET/CT values for the adenocarcinoma group
<table>
<tr><th>Sensitivity (%)</th><th>Specificity (%)</th><th>Accuracy (%)</th></tr>
<tr><td>38,8</td><td>97,4</td><td>85,7</td></tr>
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PET/CT values in the squamous cell group
<table>
<tr><th>Sensitivity (%)</th><th>Specificity (%)</th><th>Accuracy (%)</th></tr>
<tr><td>81,8</td><td>91,8</td><td>90,8</td></tr>
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<td>Principal risk factors to have occult (pN2) lymph nodes were tumour sizes ≥5 cm, pN1, adenocarcinoma and female patients. The report concluded that in tumours ≤1 cm (pT1a), surgical staging was</td>
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	tissue specimens obtained at mediastinoscopy and/or thoracotomy.			unnecessary, while adenocarcinoma and non- central cIB required a more efficient invasive staging.
Fiorelli et al (2015), Thorac Cardiovasc Surg., Italy [24] Retrospective multicenter study	901 consecutive patients with Stage I NSCLC screened with PET/CT scan undergoing surgery from January 2006 to December 2012	pN2 disease Survival of occult pN2 patients	108/901 (12%) had unexpected pN2 disease Central tumor location ($p < 0.003$), cT2a ($p < 0.0001$) and pT2a stage ($p < 0.0001$), pN1 disease ($p = 0.004$), and a standard uptake value > 4.0 (0.007) were prognostic factors of occult pN2 disease Patients with unexpected pN2 disease compared with patients with cN2 disease undergoing surgery after induction therapy presented a better median overall survival (56 versus 20 months; $p = 0.001$) and disease-free survival (46 versus 11 months; $p < 0.0001$).	The preoperative effort to discover unexpected pN2 disease in patients with clinical stage I non-small cell lung cancer is not justified, considering their good survival. indicated

De Leyn et al. (2014), Eur. J. Cardio-Thorac Surgery, Belgium [25] Systematic Review	ESTS guidelines for preoperative lymph node staging for NSCLC Systematic review into the pre-operative lymph node staging for NSCLC	Reccomandation	Preoperative mediastinal staging is advised in central tumors <3 cm, in every tumor larger than 3 cm or in CT or PET N1 nodes positivity. Instead, a systematic nodal dissection is indicated for tumors ≤3 cm, located in outer third of the lung cm and when there are no pathologic evidence on CT and or on PET or PET-CT. The choice of mini invasive technique (EBUC/EUS, mediastinoscopy or VATS) depends on local expertise to adhere to minimal requirements for staging.	
Silvestri et al (2014), Chest , USA [26] Systematic Review	ACCP guidelines for Invasive Mediastinal Staging of Lung Cancer	Reccomandation	Preoperative mediastinal staging is advised in central tumors <3 cm, in every tumor larger than 3 cm or in CT or PET N1 nodes positivity. Instead, a systematic nodal dissection is indicated for tumors ≤3 cm, located in outer third of the lung cm and when there are no pathologic evidence on CT and or on PET or PET-CT	

The update of European Society of Thoracic Surgery of 2014 [72] stated that preoperative mediastinal staging is advised in central tumors < 3 cm, in every tumor larger than 3 cm or in CT or PET N1 nodes positivity. Instead, a systematic nodal dissection is indicated for tumors ≤ 3 cm, located in outer third of the lung cm and when there are no pathologic evidence on PET-CT scan.
American College of Chest Physician [73] guidelines did not support mediastinoscopy in stage I NSCLC unless a PET scan finding is positive in the nodes. However, mediastinoscopy is indicated for central tumors, cN1 disease, or low FDG uptake of the primary tumor with N2 PET negative LNs 16 mm on CT scan.

DISCUSSION

The most of analyzed papers evaluated patients without mediastinal adenopaties on PET-CT undergoing resection. Invasive mediastinal staging was attended in all or in two/third of patients in four papers [55-58]; in half or less in two [63,64]; and in nobody patient in four [59 60 62 71]. Occult pN2 disease was < 10% in five [55 61 63-65]; between 10-16% in four [56 57 59 71]; and > 16% in four papers [60 61 65 67]. Diagnostic yield of mediastinoscopy was between 50-61% in two [55 61], and > 80% in four papers [57 58 63 64]. It missed N2 metastases because LNs were within inaccessible station or sub-centimeters [55 63]. Significant risk factors for occult pN2 disease are the SUV value of primary tumor ranging from 4 to 10 [56], central tumor [59 63 64 71], tumor larger than 3 cm [68 70], histology of adenocarcinoma [68-70] and presence of clinical hilar lymph node involvement (cN1 disease) [71]. However, the last guidelines of ESTS [54] and ACCP [53] in agreement with previous papers reported that invasive mediastinal staging is advised also for tumor < 3 cm if located in hilar region. Two papers [63 71] found that unexpected pN2 patients had a better survival than cN2 disease undergoing surgery after induction therapy.
From analysis of the literature [74-76], we can conclude that invasive mediastinal staging with mediastinoscopy, EBUS or EUS is recommended also in cN0 patients with central tumor or with peripheral tumor > 3 cm. Despite in the last years EBUS-TBNA is become the preferred approach for mediastinal sampling, mediastinoscopy or VATS remain the best options in case of lymph node with high suspicion of involvement but negative on EBUS.

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Review

Aging and cardiac autonomic control in chronic heart failure: methods and clinical implications

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A large body of evidence has been provided that cardiac autonomic control is deranged in heart failure. It is also commonly accepted that aging is characterized by several molecular and structural changes in organs and tissues, and *per se* affects cardiac autonomic control. Hence, as far as we are concerned with heart failure in the elderly, both cardiac diseases and age are likely to contribute to the autonomic dysfunction of these patients.
In the first part a brief review to the methods currently used to assess the autonomic control of the cardiovascular function in human subjects is reported. Then, major findings on the relationship between aging and cardiac autonomic indexes in normal subjects are presented. In the third part, main concept and experimental observations on autonomic dysfunction in heart failure are reviewed. Finally, some basic considerations on the relationship between aging, cardiac autonomic function and heart failure are introduced.
1. A brief review to the methods currently used to assess the autonomic control of the cardiovascular function in human subjects is reported.
2. The relationship between aging and cardiac autonomic indexes in normal subjects are presented.
3. Main concept and experimental observations on autonomic dysfunction in heart failure are reviewed.

Key words: HRV, Poincarè analysis, Fractal analysis

INTRODUCTION

A large body of evidence has been provided that cardiac autonomic control is deranged in heart failure [1]. It is also commonly accepted that aging is characterized by several molecular and structural changes [2] in organs and tissues that *per se* affect cardiac autonomic control [3-6]. Hence, both cardiac diseases and aging are likely to contribute to the autonomic dysfunction of elderly patients with heart failure.
In the first part a brief review of the methods currently used in the autonomic control assessment of the cardiovascular function in human subjects is reported. Then, major findings on the relationship between aging and cardiac autonomic indexes in normal subjects are presented. In the third part, main concepts and experimental observations on autonomic dysfunction in heart failure are reviewed. Finally, some basic considerations on the relationship between aging, cardiac autonomic function and heart failure are introduced and results from a previous study described.

METHODS OF AUTONOMIC FUNCTION INVESTIGATION IN HUMANS

Since changes in sympathetic and vagal traffic to the sinoatrial node alter the natural frequency of the cardiac

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pacemaker inducing a corresponding change in heart rate, the measurement of the latter would be the simplest way of appraising the heart autonomic control and, more specifically, the sympatho-vagal balance. The interaction among heart rate, intrinsic frequency of the pacemaker and the levels of vagal and sympathetic outflows to the heart has been model as a multiplicative relationship [7] and could be conditioned by several different factors [8-11]. Hence, given the intrinsic frequency, the net effect of the sympatho-vagal balance is expressed by the current heart rate. Unfortunately the intrinsic frequency changes between individuals and its measurement require a complete autonomic blockade. As a consequence, the measurement of heart rate provides only an uncalibrated quantification of the sympatho-vagal balance.

Beat-to-beat spontaneous fluctuations of heart rate do occur continuously in every human subject with a healthy heart and reflect corresponding fluctuations in neural traffic of efferent vagal and sympathetic nerves. The fluctuation of heart rate around its mean has commonly referred as heart rate variability (HRV). Several time- and frequency-domain indexes have been extracted in the last two decades from the HRV signal using digital signal processing techniques, and experimental evidence have been provided that known changes in sympathetic and vagal outflows to the heart, associated with physiological manoeuvres, drug administration, disease or increased risk for lethal arrhythmias, are accompanied by well-defined changes in HRV parameters [12]. It has been thus hypothesized that spontaneous cardiovascular fluctuations can be exploited to provide quantitative indexes of cardiac autonomic control mechanisms.

Time-domain HRV indexes are basically derived from direct measurement of normal-to-normal (NN) RR intervals or from the differences between them. The most common parameters obtained are the standard deviation of NN intervals (SDNN) and the root square of the mean successive squared difference of successive NN intervals (RMSSD) (task force), respectively. These measurements can be performed either on long-term (24-h) ambulatory recordings or on short-term (< 10 min) laboratory recordings. Long-term indexes, in turn, may be derived from the analysis of the overall recording, or may be calculated segmenting the entire 24-h period into consecutive small epochs (typically 5') and then averaging results over pre-defined periods of time, e.g. night and day. A depressed SDNN has consistently been found in patients after myocardial infarction (MI) and interpreted as the effect of a reduced vagal activity directed to the heart [13]. In the acute phase of a myocardial infarction the 24-h SDNN is related to left ventricular dysfunction, peak creatine kinase and Killip class [13]. Large-scale studies have shown that a depressed SDNN is also a powerful predictor of mortality and arrhythmic complications in post-MI patients, independently of other well-established risk stratification markers such as left ventricular ejection fraction, ventricular ectopic activity and presence of late potentials [13].

Frequency-domain methods aim to identify and estimate major rhythms hidden into the apparently erratic behaviour of the HRV signal. These methods are mostly used in short-term recordings, typically ranging from 2 to 5 min [7 12-14]. Three rhythms or spectral components are commonly detected: the very low frequency (VLF) rhythm in the range: 0.01-0.04 Hz, the low frequency (LF) rhythm in the range 0.04-0.15 Hz and the high frequency (HF) or respiratory rhythm in the range 0.15-0.4 Hz. Automatic signal processing procedures provide both the central frequency and power of these spectral components [15]. Graded orthostatic tilt, which is known to be associated with sympathetic activation, typically causes an increase of the LF component and a simultaneous decrease of the HF component. This displacement of power from one component to the other is well correlated with the angle of tilt [16]. Pharmacological blockade of beta-adrenergic receptors causes an unbalance of the power content in favour of the HF component, whereas muscarinic receptor blockade causes a prevalence of the LF over the HF component [12]. These findings, together with several experimental observations from animal studies, led to the conclusion that in spontaneous rhythms the analysis of the HRV signal, more appropriately, the relative power of the LF and HF components expressed as normalized powers or LF/HF power ratio, are capable of providing quantitative markers of the sympatho-vagal balance [12]. More recently, several investigators have shown that heart rate fluctuations share some basic properties with nonlinear dynamics and chaotic determinism [16-19].

Complex interactions of hemodynamic, electrophysiological and humoral variables as well as reflex and central regulatory mechanisms involved in cardiovascular function [20-23] are thought to determin Nonlinear phenomena involved in the genesis of HRV.

It has been speculated that the HRV analysis based on methods of nonlinear dynamics may provide valuable information for the physiological interpretation of HRV and for prognostic stratification of cardiac disease patients [24-26].

The parameters most often used to measure nonlinear properties of HRV include 1/f scaling of Fourier spectra, D_2 correlation dimension, Lyapunov exponent, Kolmogorov entropy, H scaling exponent and Coarse Graining Spectral Analysis. For data representation, Poincarè plots, low dimension attractor plot, singular

value decomposition, and attractor trajectories have been used. Although all these techniques are in theory powerful tool for the analysis of HRV, their practical usefulness is still controversial. Indeed, most attempts to apply nonlinear dynamics techniques to real data have provided either trivial results or intriguing speculations. Moreover, some basic methodological issues, such as the confounding effect of non-stationarity in the observed HRV time series, still need to be solved. Finally, the general requirement of long-term recordings for these methods prevents their application to laboratory data [27].

Two nonlinear techniques have recently gained great interest due to remarkable results in clinical studies: the 1/f scaling of Fourier spectra and Poincarè plots [28].

The former method has recently been applied in the prognostic assessment of patients with recent myocardial infarction and patients with heart transplants, and it has been shown that power law regression parameters are excellent predictors of death of any cause or arrhythmic death and predict these outcomes better than the traditional power spectral bands [29 30].

The Poincarè plot method has been used by some investigators in patients with mild to moderate chronic heart failure, showing an independent prognostic value and identifying increased risk for all-cause cardiac death [31]. This method consists in constructing HRV maps by plot of each RR interval against the subsequent one. These maps allow detecting patterns resulting from non-linear processes that may not be detectable by other classical methods of analysis. A simple example is given by sudden changes in the RR interval, which would appear in the power spectrum as wide-band noise. The major limitation of the traditional approach to the analysis of Poincarè plots is the visual classification of plot shapes and the manual measurement of the maps. To overcome this limitation our group has developed new algorithms for automatic morphological quantification of the plots [32 33], which allows to extract relevant parameters like length, wideness and area of the bi-dimensional plots and number of peaks and radii of inertia of the three-dimensional maps (Fig. 1).

Arterial baroreceptors play a major role in controlling the cardiac autonomic nerves activity through quick adaptation to changes in pressure and tissue perfusion in response to daily activities. The evaluation of baroreflex sensitivity has become a widely used clinical tool since it has been recognized that vagal and sympathetic control is deeply deranged in several cardiac diseases, and that changes in sensitivity of heart baroreflex control may be highly relevant for the outcome of these patients [34].

Among the various quantitative methods developed to estimate baroreflex sensitivity, the most widely used technique is the measure of the heart rate response to the injection of a vasoactive drug free of cardiac action. Baroreceptors stimulation with the vasoconstrictor agent phenylephrine has become the reference for the clinical evaluation of baroreflex sensitivity. However, other techniques such as the neck chamber have been largely used, mainly for research purposes [35]. More recently, several methods have been devised to estimate

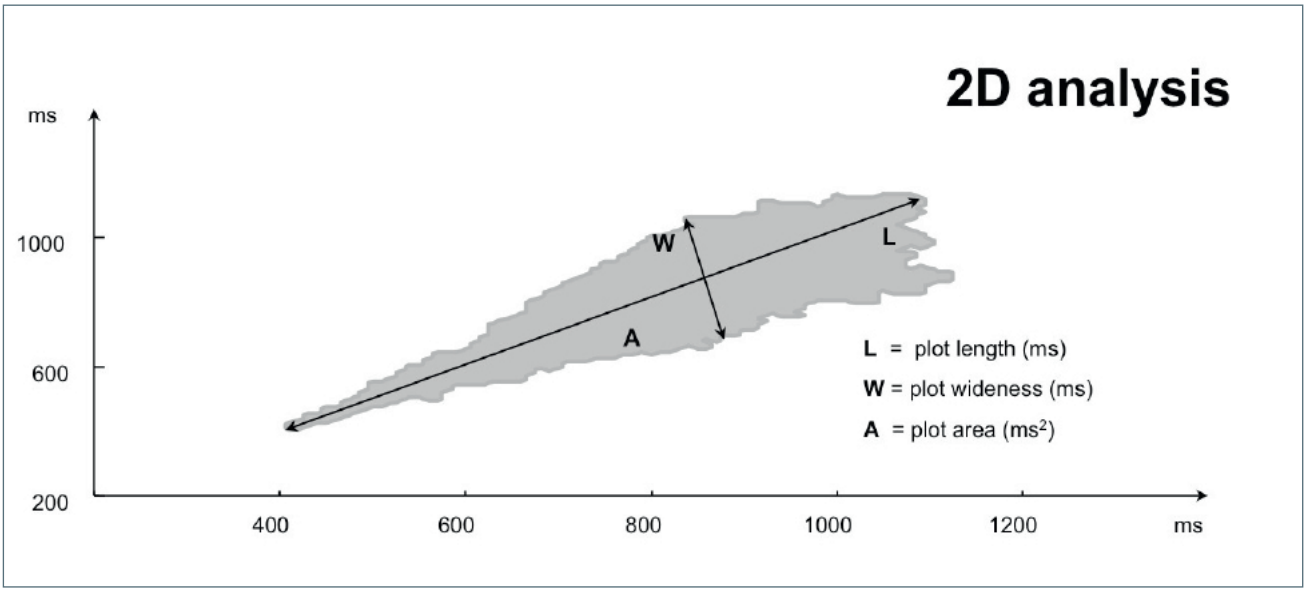

Figure 1. New algorithms for automatic morphological quantification of the plots, which allows to extract relevant parameters like length, wideness and area of the bi-dimensional plots and number of peaks and radii of inertia of the three-dimensional maps.

the baroreflex gain by the analysis of spontaneous beat-to-beat fluctuations of systolic arterial pressure and of related changes in the RR interval, thus avoiding the need of drug injection. Two basic approaches have been proposed and validated: the time-domain approach, better known as the sequence method, and the frequency domain approach, which exploits LF and HF oscillatory components in arterial pressure as "stimuli" for the baroreceptors**,** and measures related changes in corresponding oscillatory components of the RR interval [35 36].

AGING AND THE AUTONOMIC NERVOUS SYSTEM

A full knowledge of age-related changes of the autonomic nervous system in humans is still lacking. There is general consensus that aging is accompanied by increased plasma norepinephrine concentration and decrease of a) cardiac norepinephrine stores, b) affinity of beta-receptors stores, c) inotropic and chronotropic response to beta-agonists and d) baroreflex sensitivity [37]. Supine resting heart rate does not change significantly with age, whereas a significant lengthening of the RR interval has been observed both in the seated and in the standing posture of elderly [3 38]. HRV has consistently been found to decrease with age, independently of aerobic capacity and body mass index [3 38-40]. Time indexes of HRV show a pattern of change with age, which is a dependent measure. SDNN, for instance, decreases very gradually with aging with a quadratic regression pattern and a 40% reduction between 20 and 95 years [40]. Conversely, RMSSD decreases in the same length of time by about 60% [40]. The presence of a blunted baroreflex response together with a decreased HRV and a reduced heart response to atropine have been interpreted as evidence of decreased parasympathetic activity in elderly people. Postural change from supine to standing produces significant variations in heart rate and HRV, but this effect is blunted in elderly with respect to young people [38]. Since aging makes an increase in peripheral vascular resistance and a decrease in peripheral vascular capacitance, it is likely that baroreceptor-mediated modifications in HRV in response to posture-induced changes in vascular dynamics are reduced in elderly persons as a consequence of reduced demand made upon baroreceptors. Some investigators have shown that elderly people have less movement of thoracic blood into lower extremities in response to lower body negative pressure, suggesting that during postural change a similar phenomenon could occur, thus reducing the need for baroreceptor-mediated pressure regulation [41 42]. However, a reduction of the sensitivity of the baroreflex arc *per se* with aging cannot be excluded, contributing both to the reduced response of heart rate and HRV to postural change as well as to the reduced supine resting HRV.

Using spectral indexes of HRV, absolute powers in the LF and HF bands have been found to be significantly and negatively correlated with ageing [43]. Among all variables the ln(LF) parameter is the best correlated with age with a coefficient of determination which explain more than 15% of variability by aging [43]. The normalized LF power and LF/HF power ratio, but not the normalized HF power, were found to correlate with age. However, no significant changes were detected, especially in men, until age 60 years [43]. Although the age-related decrease in HRV has been commonly attributed to a decline in parasympathetic activity, the reduction in normalized LF power with increasing age suggests that sympathetic activity may also drop with age. Conversely, the fall in absolute LF power might simply reflect the decline of baroreflex sensitivity.

As depressed HRV has been proposed as a marker of a number of pathological conditions and of increased risk of mortality in cardiac patients, the use of HRV for predictive purposes must take in account the confounding effect of age. Umetani et al. [40], analysing 24-h HRV time-domain indexes in 260 healthy subjects, found that either the SDNN or RMSSD of subjects > 65 years old fell below published cut-points for increased risk of mortality in respectively 25% and 12% of them. In the same study the range of variation of all HRV measures, defined as 95% confidence limits, was wider in young subjects and narrows with increasing age, reflecting a decrease in interindividual differences over time.

AUTONOMIC DYSFUNCTION IN HEART FAILURE

Heart failure is commonly characterized by a prominent neurohormonal excitation which appears as increased sympathetic activity, increased circulating levels of norepinephrine, vasopressin and renin, withdrawal of parasympathetic activity and impaired baroreflex gain [1 44-46]. Evidence of increased central sympathetic outflow has been provided by direct recording of nerve firing in sympathetic nerves innervating muscular or cutaneous vascular beds, and by correlating the level of this firing with plasma norepinephrine levels [47]. Caution, however, should be exerted in interpreting plasma norepinephrine concentrations as measure of sympathetic nerve traffic in humans, since it actually represents the balance between norepinephrine spillover (i.e. the neurally released norepinephrine) and its clearance. When plasma norepinephrine spillover is measured separately from norepinephrine clearance, the former is on average double in heart failure patients compared to control

subjects and the latter is reduced by about a third [48]. Indirect evidences of generalized sympathoneural activation in decompensated congestive heart failure are represented by clinical observation of tachycardia, tachypnea, diaphoresis, pallor, agitation and renal sodium retention [49 50].

In a similar way only indirect evidence has been provided on parasympathetic withdrawal in heart failure. Besides the original demonstration by Eckberg et al. of a defective parasympathetic control of heart rate [51], the observation of a reduced bradycardic response to the pressor stimulus of phenylephrine has been interpreted as the effect of reduced vagal outflow to the heart. However, as increased sympathetic activity may interfere with the ability to increase vagal activity, a more realistic interpretation of depressed baroreflex gain in heart failure is to be secondary to a concomitant and opposite alteration in the activity of the two autonomic limbs [52].

The generalized sympathetic activation and parasympathetic withdrawal in heart failure have been attributed to alterations in inhibitory and excitatory influences on vasomotor neurons. In normal subjects afferent inputs from arterial baroreceptors as well as from cardiopulmonary mechanoreceptors exert a major inhibitory influence on sympathetic outflow, whereas discharge from muscle metaboreceptors are major excitatory inputs [53 54]. The vagal limb of the baroreceptor heart rate reflex is also responsive to arterial baroreceptor afferent input. At rest the net effect of these competing influences is characterized by a relatively low sympathetic activity. In heart failure the principal stimuli to baroreceptors (mean pressure, pulse pressure and rate of increase of blood pressure) are blunted and the sensitivity of cardiopulmonary mechanoreceptors diminishes, reducing inhibitory input. Moreover, excitatory input may originate from arterial chemoreceptors and skeletal metaboreceptors. The net response to this shift in balance between inhibitory and excitatory afferent inputs is a generalized increase in basal sympathetic outflow, parasympathetic withdrawal and impaired regulation of heart rate and vascular resistance.

Several investigators have attempted to assess the autonomic dysfunction of heart failure patients through analysis of HRV [55 56].

A consistent finding has been that HRV measured either in the time or frequency domain, in short-term or long-term recordings, is markedly depressed in heart failure [1 57 58], a finding, which has been interpreted as the effect of impaired parasympathetic control of heart rate. The amount of heart rate variability is closely and negatively related to the degree of sympathoexcitation as expressed by muscle sympathetic nerve activity and plasma norepinephrine [59]. When HRV in heart failure patients is assessed through spectral methods and compared to normal subjects, the distribution of the variability over frequency invariably shows a shift from the LF and HF band to the VLF band [57]. Hence, all oscillatory components of HRV in heart failure are depressed with respect to normal controls but, at the same time, VLF oscillations are proportionally much higher then the other spectral components. The presence of a reduced HF component supports the notion of parasympathetic withdrawal in heart failure patients, as this component is almost entirely vagal-mediated. The LF component shows two typical patterns. In some patients it is predominant over the HF component, suggesting, as expected, a shift of the sympathovagal balance in favor of sympathetic activation [60]. In other patients the HF component is still low but the LF component has almost disappeared [57]. These patients are characterized by a greater severity of the disease, including a higher NYHA class, a more depressed left ventricular function and a higher degree of sympathoexcitation as evidenced by higher levels of plasma noradrenaline. This paradoxically low LF component in presence of a pronounced sympathetic activity has been explained by the concept that in the more severe stages of the disease an abnormally high sympathetic tone may be capable of "saturating" the sinus node response, making it almost insensitive to modulations of this tone [60]. However, in chronic heart failure patients, spectral analysis of resting muscle sympathetic nerve activity and RR interval has recently shown a close coherence between the variability patterns of the two signals [61]. A consistent finding of this and other studies is that patients with very depressed or absent LF component have the worse prognosis [60 61].

In recent years, the increasing evidence of association between various respiratory and cardiovascular diseases [59], and the simultaneous recording of cardiovascular (ECG, arterial blood pressure) [62] and respiratory (lung volume, SaO_2) signals in patients with heart failure has disclosed new important information on respiratory abnormalities of these patients and on related implications on cardiovascular regulation [63-71]. These respiratory abnormalities are typically characterized by a smooth rise and fall in ventilation with cycle lengths ranging from about 25 s to 100 s (0.01¸0.04 Hz) and are commonly referred to as periodic breathing, or, usually when separated by apnea, Cheyne-Stokes respiration. Although the phenomenon of periodic breathing has been studied mostly during sleep, recent investigations have shown that it has a high prevalence in awake patients, ranging from 25% to 66% during recordings in controlled laboratory conditions in patients with mild to moderate severity of the disease (New York Heart Association class I to III) [72 73]. The ventilatory oscillation is

accompanied by a synchronous oscillation of arterial O_2 saturation, which, especially in the more accentuated forms of periodic breathing, brings about marked cyclic desaturations. As a consequence, a chemoreceptor-induced sympathetic excitation results, which adds to an already existing condition of sympathetic predominance [61 74]. Moreover, the cyclic change in ventilation is also accompanied by a phase-linked oscillation of heart rate and arterial blood pressure in the VLF band, which dominates the overall fluctuating pattern of these signals [75]. These findings clearly indicate that during periodic breathing a deep simultaneous involvement of the respiratory and cardiovascular systems does take place. Moreover, they also point out that the use of HRV for the assessment of autonomic cardiovascular regulation and for prognostic evaluation in heart failure patients must take into account the confounding effect of periodic breathing [73].

Evidence has been provided that alterations in sympathetic and parasympathetic outflows to the sinoatrial node in heart failure can be identified by analysis of Poincarè plots shape from 24-h heart variability recordings [76]. While normal subject typically show a comet-shaped pattern, indicating an increasing variability at lower heart rates, heart failure patients show three main patterns: 1) a contraction in the plot's length with a torpedo-shaped pattern, resulting in a reduced distribution of the whole heart rate dispersion, 2) a fan-shaped pattern, with a great dispersion on a narrow range of frequencies and 3) a complex-shaped pattern, consisting of a thin core area and several clusters of points (Fig. 2).

The mechanisms responsible for these strikingly different patterns are not entirely clear and are currently under investigation in our laboratory. It has been suggested that the core of the pattern is the result of sympathetic influences, whereas the increased dispersion at longer RR intervals reflects parasympathetic

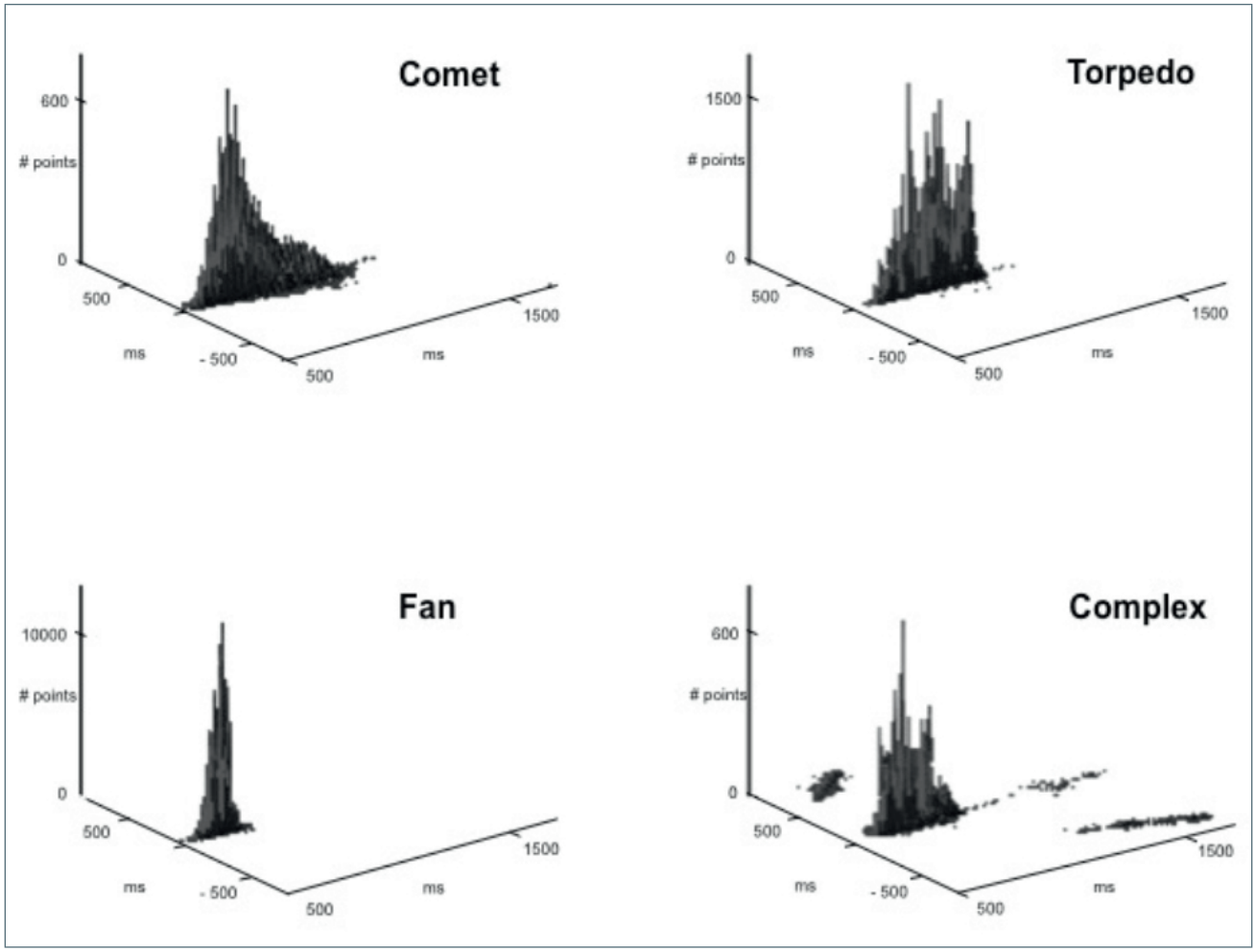

Figure 2. Alterations in sympathetic and parasympathetic outflows to the sinoatrial node in heart failure identified by analysis of Poincarè plots shape from 24-h heart variability recordings.

activity, respiratory sinus arrhythmia or sleep state. The torpedo-shaped pattern seems the one closer to the normal pattern, whereas the complex- and fan-shaped pattern reflect a greater disturbance in autonomic cardiac regulation. We have developed a set of morphological quantification indexes in order to provide an objective assessment of Poincarè maps. These indexes are characterized by an excellent short- and long-term reproducibility in stable chronic heart failure patients, indicating that they constitute reliable measures suitable to be used in the clinical setting [33]. Among the overall set of 2-dimensional and 3-dimensional indexes, the latter are closely and independently related to plasma norepinephrine levels in patients with advance heart failure [77 78].

AGING AND AUTONOMIC FUNCTION IN HEART FAILURE

We have seen so far that that both aging and heart failure affect the autonomic regulation of the cardiovascular system and that marked changes in heart rate variability follow the progression of age and of disease severity. It thus appears that when heart failure develops or progresses in the elderly both factors contribute concurrently to the deterioration of the autonomic function and their effects tend to be confounded. Although intuitively the effect of aging and heart failure would sum each other, there are no definite proofs that they are additive. In general, studies on the relationship between aging and heart failure are scanty. It is well known that impaired cardiac beta-adrenergic receptor (beta-AR) signalling and function represents a hallmark underlying mechanism of chronic heart failure (HF) pathophysiology, characterized by a beta-AR downregulation and desensitization of both beta-AR and beta-AR subtypes [79 80].

We recently analyzed time- and frequency-domain as well as Poincarè plot indexes of HRV from 24-h ambulatory recordings of 41 chronic heart failure patients (NYHA class III-IV) and compared the results with those from 59 patients with coronary artery disease without signs or symptoms of heart failure. Patients were divided according to the standard cut-off age of 65 years. Results are given in Table I. It can be noticed that in subjects under 65 years with the exception of the HF power (in absolute units) and the Width parameter of Poincarè plots, all indexes of heart rate variability are significantly reduced in HF compared to CAD patients, in agreement with the notion of depressed variability in heart failure. In patients over 65 years almost all heart rate variability indexes except the LF power in normalized units do not change significantly with respect to younger subjects in both groups. Peak number and mean peak distance of Poincarè plots are the only variability indexes, which differentiate heart failure patients from CAD patients in both age groups. These results suggest that cardiac autonomic regulation in chronic heart failure and CAD patients over 65 years does not change in a clearly detectable way with respect to the same patients under 65 years old. As expected, chronic heart failure patients

Table I. Relationship between age and major time-domain (Mean RR, SDNN), frequency-domain (lnLF, lnHF, LF_{NU}) and Poincarè plot (Length, Width, 3D peak number, Mean peak distance) indexes of heart rate variability from 41 chronic heart failure patients and 59 patients with coronary artery disease without sign or symptoms of heart failure.

	Age < 65 years		Age ≥ 65 years	
	CHF	CAD	CHF	CAD
N	22	35	19	24
Age (years)	57 ± 6	37 ± 15**	70 ± 4 ††	73 ± 6 ††
EF (%)	24 ± 7	63 ± 5**	26 ± 6	55 ± 7** ††
Mean RR (ms)	790 ± 119	833 ± 168	834 ± 198	985 ± 157* ††
SDNN (ms)	102 ± 31	143 ± 40**	110 ± 63	133 ± 41
lnLF (ln(ms^2))	5.93 ± 0.46	6.38 ± 0.37*	5.8 ± 0.8	5.97 ± 0.53
lnHF (ln(ms^2))	5.47 ± 0.44	5.7 ± 0.4	5.49 ± 0.94	5.56 ± 0.5
LF_{NU} (%)	60 ± 19	65 ± 15**	57 ± 36	59 ± 32 ††
Length (ms)	139 ± 43	228 ± 58**	145 ± 81	193 ± 50
Width (ms)	28 ± 15	32 ± 9	45 ± 45	31 ± 12
Peak number	18 ± 8	30 ± 12**	16 ± 6	25 ± 6**
Mean peak distance (ms)	4.5 ± 4.2	12.7 ± 6.9**	3.94 ± 4.02	8.34 ± 6.8**

Results are expressed as meanSD. EF: ejection fraction; SDNN: standard deviation of normal-to-normal RR intervals; LF: absolute power in the LF band; HF: absolute power in the HF band; LF_{NU}: LF power in normalized units; ln: natural logarithm.f

* $p < 0.01$ *vs* CHF; ** $p < 0.001$ *vs* CHF

† $p < 0.05$ *vs* < 65 years; †† $p < 0.001$ *vs* < 65 years

have depressed variability compared to CAD patients that is most consistently described by the peak number and mean peak distance of the Poincarè plots.

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Review

Recent advances in basic and clinical research on the prevention and treatment of the metabolic syndrome and related disorders by the use of olive polyphenols

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Type 2 diabetes mellitus (T2DM) has been defined, together with obesity, the XXI century epidemic and, together with cardiovascular disease, concurs to define a pathological condition known as metabolic syndrome. T2DM can be prevented by an adequate lifestyle and treated, in the preclinical stage and at the onset of the clinical signs, by a diet rich in olive tree polyphenols, in addition to the pharmacological therapy. Studies on animals and humans suggest that olive tree and other plant polyphenols contribute significantly to most of the beneficial effects associated with the Mediterranean diet including reduced cardiovascular disease, T2DM, cancer and aging-associated neurodegeneration. These studies suggest the possible use of plant polyphenols in dietary supplements as nutraceuticals useful against the metabolic syndrome and related conditions, particularly T2DM. The present review summarizes the scientific data on the healthy virtues of the olive polyphenols that support such conclusion.

Key words: Oleuropein, Olive leaf extract, Olive tree polyphenols, Metabolic syndrome, Age-related dysmetabolism, Type 2 diabetes

INTRODUCTION

In the last two decades, the concept of metabolic syndrome has gained widespread consensus as a powerful hypothesis that unifies the metabolic factors underlying the development of both cardiovascular disease, fatty liver disease and type 2 diabetes mellitus (T2DM). In recent years, the incidence of these pathological conditions has involved an increasing number of young people, even though, together with cancer, they represent the main clinical emergence in aged people. However, in addition to age, the relevance of lifestyle, including physical exercise and alimentation, in the etiopathogenesis of these diseases has gained momentum in the medical community. In particular, T2DM, defined together with obesity (Diabesity) as the XXI Century epidemic, is a so-called wellness disease that can be prevented by an adequate lifestyle and treated, in the preclinical stage and at the onset of the clinical signs with the pharmacological therapy possibly implemented with a diet rich in plant polyphenols.

T2DM accounts for about 90% of diabetes cases worldwide and in the past 50 years its incidence in the world has increased significantly, and in parallel with the growth of obesity, from 30 million in 1985 to 135 million in 1995 and 217 million in 2005. It has been calculated that in 2013 there were approximately 368 million people diagnosed with the disease compared to around 30 million in 1985 [1]. Therefore, the World Health Organization has recognized this disease as a global epidemic. In Italy, T2DM has been calculated to affect about 5 million people, of which around 3.5 diagnosed. The insurgence of T2DM is mainly caused by a combination of factors including diet, lifestyle, endocrine

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anomalies, female sex and genetic predisposition [2 3]. Other potential diabetogenic factors include insufficient sleep and the mother's nutritional status during pregnancy that can induce fetal abnormalities through epigenetic mechanisms [4]. As far as lifestyle is concerned, the risk of developing T2DM is influenced by several factors, including obesity (a body mass index > 30) [5], reduced physical activity [6] and inaccurate diet (excessive consumption of sugar, excess of saturated and trans fatty acids, reduced intake of unsaturated fatty acids) [7]. In most cases, the predisposition to T2DM is genetically based and involves numerous genes (over 36 recognized to 2011), each one contributing partially to the disease. Most of the diabetes-related genes are involved in physiological aspects relative to insulin secreting pancreatic beta cells [8]. Many genes, alleles and allelic combinations favor the onset of T2DM, the TCF7L2 allele being apparently the most important; these include genes belonging to the lipases family, different adrenaline receptors and several alleles of the insulin receptor [9].

T2DM results from either insufficient insulin production by the pancreatic beta cells and a condition of insulin resistance. The latter consists of a reduced response by the body cells, particularly in the liver and the adipose tissue, to the insulin action [8 10]. Other potentially important abnormalities associated with T2DM and insulin resistance (Fig. 1) consist of (i.) increased lipid deposits in fat cells, (ii.) a condition of dyslipidemia and liver disease/nonalcoholic steatohepatitis, (iii.) the lack, or low levels, of hormones and cytokines such as testosterone, estrogen, insulin-like growth factors, etc., that increase insulin sensitivity [9], (iv.) the presence of elevated levels of other hormones that inhibit the action of insulin (adrenocortical hormones, glucagon, adrenalin), and (v.) an improper regulation of metabolism in the central nervous system. T2DM is a chronic condition associated with a ten years shorter life expectancy as compared to the average [11]. This reduction is, in part, due to various T2DM-related complications including the increased risk of cardiovascular diseases, cognitive dysfunction and dementia (Alzheimer's disease, vascular dementia) and blood circulation problems.

A number of pharmacological therapies are presently available to treat T2DM with various success. In addition to these, in recent years the validity of the use of polyphenols-enriched plant extracts has increasingly gained attention in the medical and scientific communities. This review focuses recent data highlighting the potential use of olive oil and olive polyphenols as natural tools useful to prevent and to combat the metabolic syndrome and T2DM, its main related condition, in addition to the pharmacological therapy.

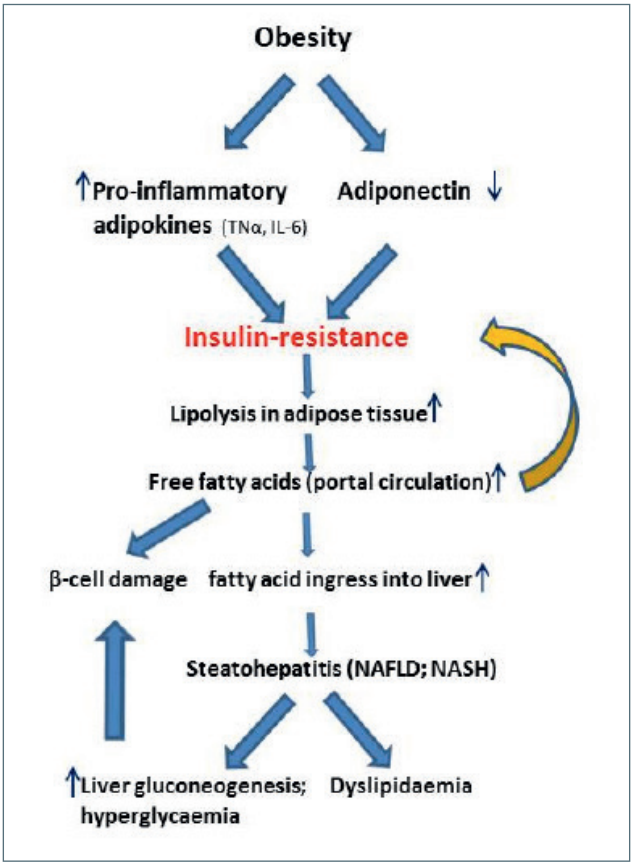

Figure 1. Schematic view of the main metabolic effects of insulin resistance in T2DM (Modified from http://medmedicine.it/articoli/73-endocrinologia-e-metabolismo/diabete-mellito-di-tipo-2).

T2DM AND THE METABOLIC SYNDROME

In recent years an unifying theory was established on T2DM, central obesity and cardiovascular disease (CVD). All these conditions appear to be linked into the concept of *metabolic syndrome*, but the underlying causes are not fully described [12]. Figure 2 schematically describes a current hypothesis on metabolic syndrome pathogenesis.

Prevention and treatment of T2DM

T2DM is a pathological condition closely related to the metabolic syndrome and its onset may be delayed or prevented by precautions such as a proper nutrition and a regular exercise that can reduce by over one half the risk of disease in healthy people.

Diet and exercise, either alone or in combination with drug therapy may also decrease the risk of developing T2DM in patients with impaired glucose tolerance. At these initial stages, the interventions on lifestyle appear more effective than the pharmacological treatment of first choice with metformin [13].

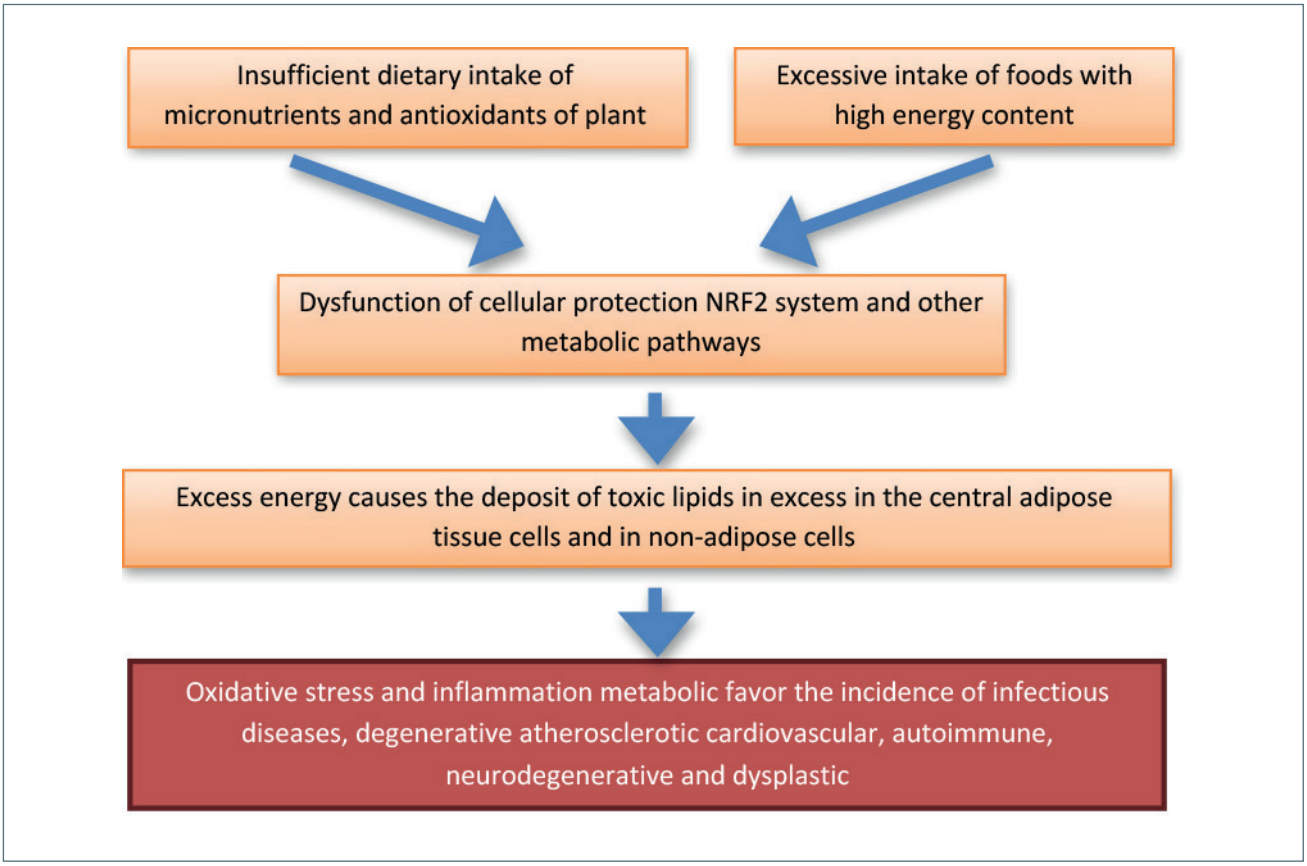

Figure 2. Scheme of the pathogenic process underlying the metabolic syndrome.

When considering the metabolic syndrome, T2DM management focuses to lifestyle interventions, to the reduction of other risk factors for CVD such as hypertension, hypercholesterolemia, microalbuminuria and to the maintenance of correct blood sugar values; in this respect, a proper diet combined with physical activity is considered essential [14]. It is also important that the weight-reducing diet is characterized by a low glycemic index. In patients with mild diabetes, in which food and lifestylechanges have not improved the glycemic control, the pharmacological treatment is also taken into account. Various classes of hypoglycemic agents are available as anti-diabetics (biguanide, glinides, thiazolidinediones, acarbose, sulfonylureas, insulin) that should always be used in combination with a proper lifestyle (Fig. 3). In this regard, plant polyphenols-based nutraceutical supplements including epigallocatechin gallate, curcumin, resveratrol and oleuropein can be used for their general power to prevent pathological states associated with the metabolic syndrome, including T2DM and neurodegeneration, and to complement the pharmacological therapy. In this respect, oleuropein and other olive polyphenols appear of significant interest (see below).

NUTRITONAL AND HEALTHY PROPERTIES OF OLIVE (OLEA EUROPAEA) POLYPHENOLS

Food polyphenols

The phenolic compounds contained in plant foods, whose progenitor is considered hydroxybenzene (C_6H_5OH), also known as phenol or carbolic acid, are a heterogeneous mixture of substances chemically derived from aromatic hydrocarbons by substitution of one (phenols) or more (polyphenols) hydrogen atoms with hydroxyl groups. Polyphenols are found mainly in foods of plant origin, while their presence in food of animal origin is occasional, resulting from the assumption of plant foods by animals; tyrosine and its metabolites (catecholamines, thyroid hormones and several intermediates of melanin synthesis) are the only important exceptions.

Over 10,000 different compounds of phenolic nature are known in plants, where they play important functions, such as defense as repellants for herbivores and insects, protection against the ionizing effects of the ultraviolet radiation, attraction of pollinators, elimination of microbes and insects (phytoalexins) and inhibition of the

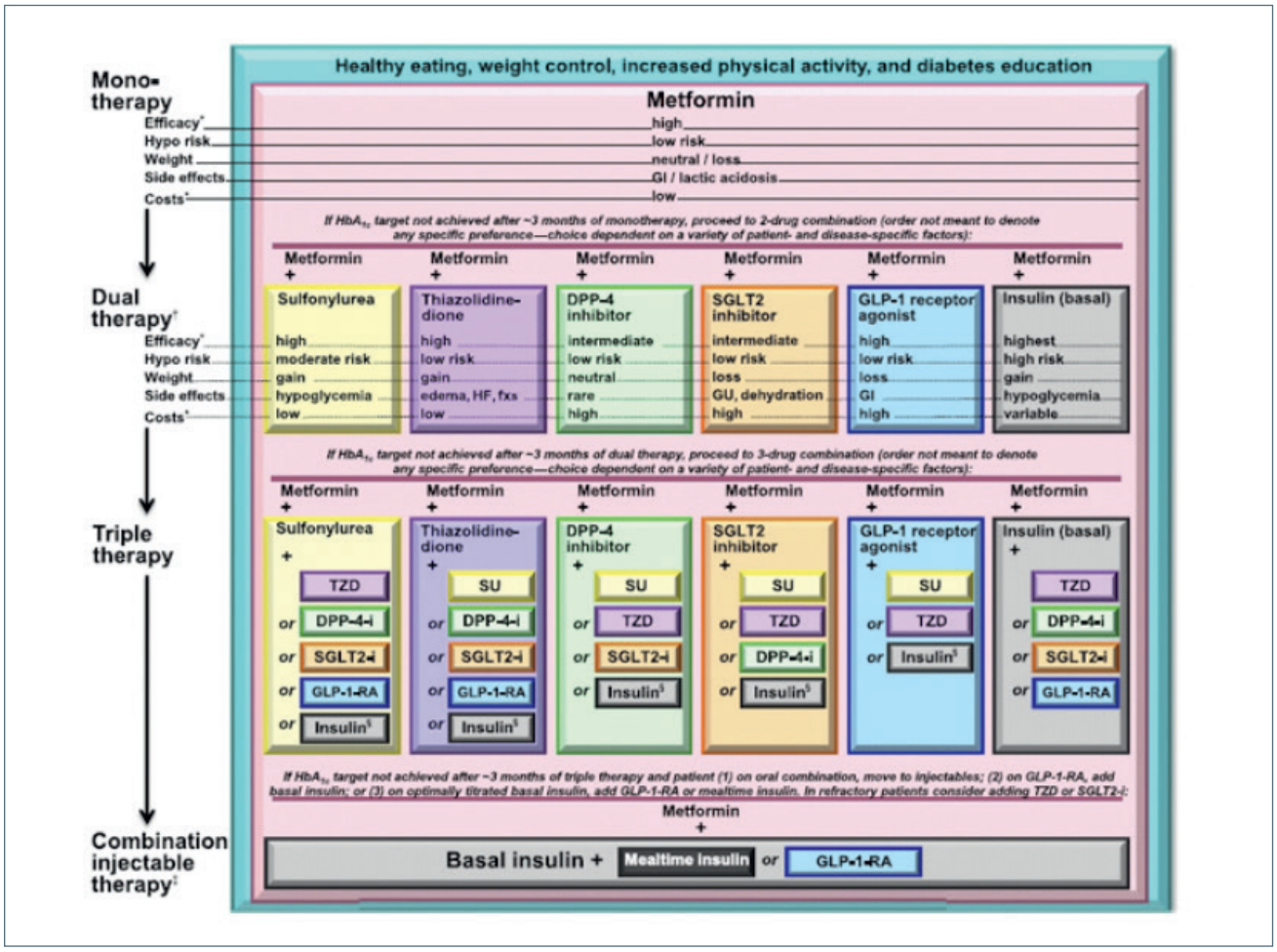

Figure 3. Algorithm for the T2DM therapy (Taken from http://care.diabetesjournals.org/content/38/1/140).

growth of competing plant species. Figure 4 shows the molecular structures of different polyphenols of plant origin with claimed beneficial properties against aging and many aging-related diseases, including cancer, neurodegenerative, immunological, metabolic, cardiovascular and inflammatory, diseases. These properties, are known since long time and presently are supported by many experimental data, both in animal models and in humans (reviewed in 15). The polyphenols found in foods characteristic of the Mediterranean diet (MD), such as olive oil and red wine, have been particularly studied in relation to the beneficial properties of this alimentary regimen and to their claimed efficacy against several chronic degenerative diseases (see below).

Olive tree polyphenols

Olive oil, obtained by pressing the drupes produced by *Olea europaea*, can be considered a basic ingredient of the MD and, more generally, of the Mediterranean lifestyle (Fig. 5). An important aspect, often not adequately considered by consumers, is that oil freshness influences considerably the organoleptic, nutritional and healthy profile of an olive oil. The spicy flavor of a fresh olive oil decreases with aging because the polyphenols responsible for it are increasingly lost due to oxidation and to slow sedimentation of the minute water droplets in suspension in the oil phase where they are largely contained. In addition to the components found in major amounts, olive oil and olive leaf extracts contain many other substances at low concentrations. These include phenols (*tyrosol* and *hydroxytyrosol*) together with two main polyphenols, *oleuropein* and *oleocanthal* (Figs. 5 and 6) both in the glycated form or as aglycones. Olive oil also contains carotenoids, tocopherols (mainly α-tocopherol) and tocotrienols, catechins, terpene alcohols, phytosterols, etc. The presence of tocopherols and polyphenols gives the oil significant antioxidant and "anti-aging" properties in part due to their ability to detoxify free radicals, while the presence of some phenols (hydroxytyrosol) confers antiplatelet and

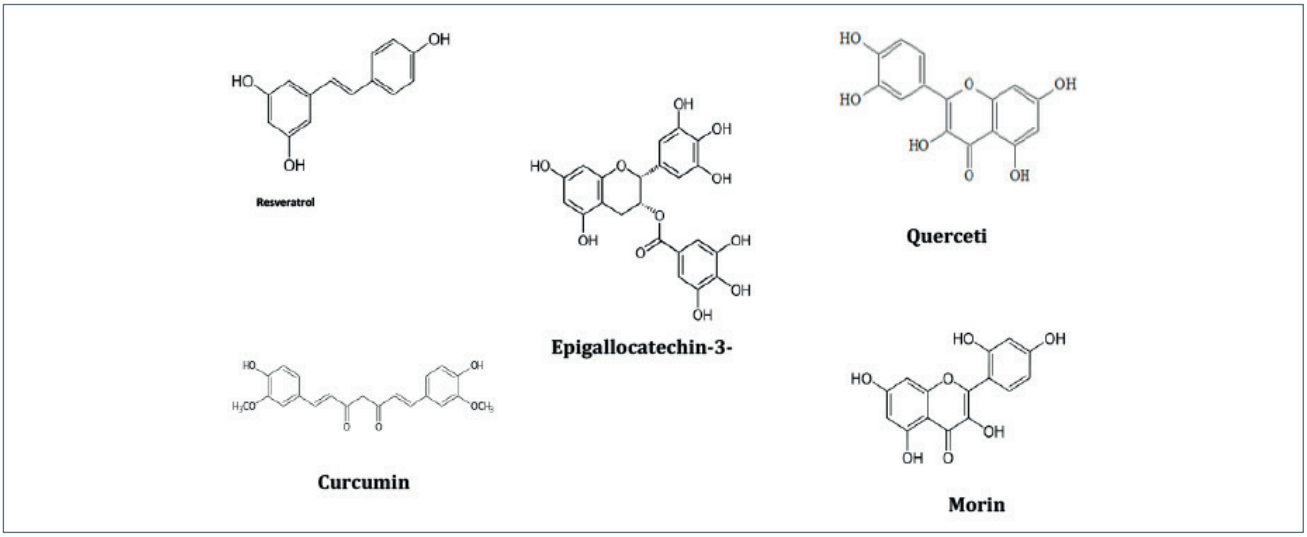

Figure. 4 Molecular structures of some common plant polyphenols.

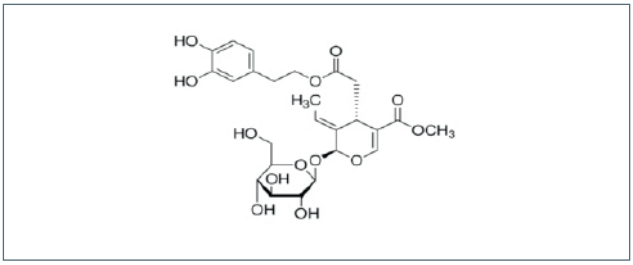

Figure. 5 Molecular structure of oleuropein glycoside. It is plentiful in leaves and green olive drupes. In olive oil and in ripe olives the aglycone form prevails.

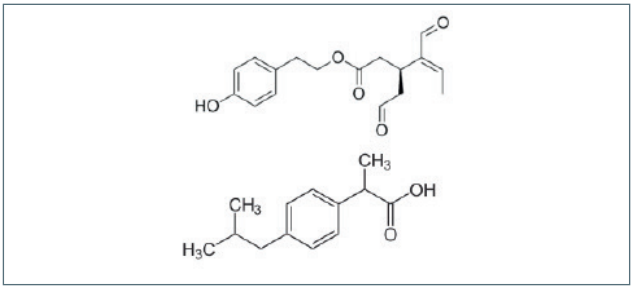

Figure 6. Molecular structure of oleocanthal (top) and ibuprofen (bottom).

anti-inflammatory power. In this regard, recent research has associated the mild anti-inflammatory activity of olive oil to the content of oleocanthal, the main responsible of the spicy flavor of fresh olive oil, whose structure is similar to that of ibuprofen, a widely used anti-inflammatory drug (Fig. 6). Accordingly, oleocanthal has been proposed to act similarly to ibuprofen inhibiting the activity of cyclooxygenases, enzymes involved in the inflammatory response [16].

The phenolic content in the olive oil may vary considerably as an effect of many factors. These include olive variety and degree of ripeness, climate, cultivation, oil production techniques, together with time and mode of storage. The polyphenols content in olive oil decreases remarkably with oil aging, mainly due to oxidation; oil separation from the polyphenol-rich minute water droplets by filtration or precipitation also reduce considerably polyphenol content. At the best conditions, the highest concentration of total polyphenols in olive oil reaches values of 600-800 mg/kg. Fig. 7 reports the different content of polyphenols in some Italian olive cultivars.

Oleuropein, hydroxytyrosol and oleocanthal are among the main components of the olive leaves extracts and are considered responsible for the beneficial properties of the latter. The benefits of a diet rich in olive oil and of the assumption of olive leaf extract-based nutraceuticals have been highlighted in recent years by many clinical studies and population surveys carried out on Mediterranean or non-Mediterranean populations. However, the clinical trials with polyphenol-enriched olive extracts are scarce and have mainly been carried out on small cohorts of patients, which reduces the statistical significance of the reported results (see later).

The healthy properties of olive polyphenols

The MD and the intake of olive leaf extract-based nutraceuticals have been associated with reduced risk of CVD, as shown by the *Seven Countries Study*, performed since early 1960s, an important contribution to our knowledge on the relationship between consumption of monounsaturated fatty acids in a Mediterranean

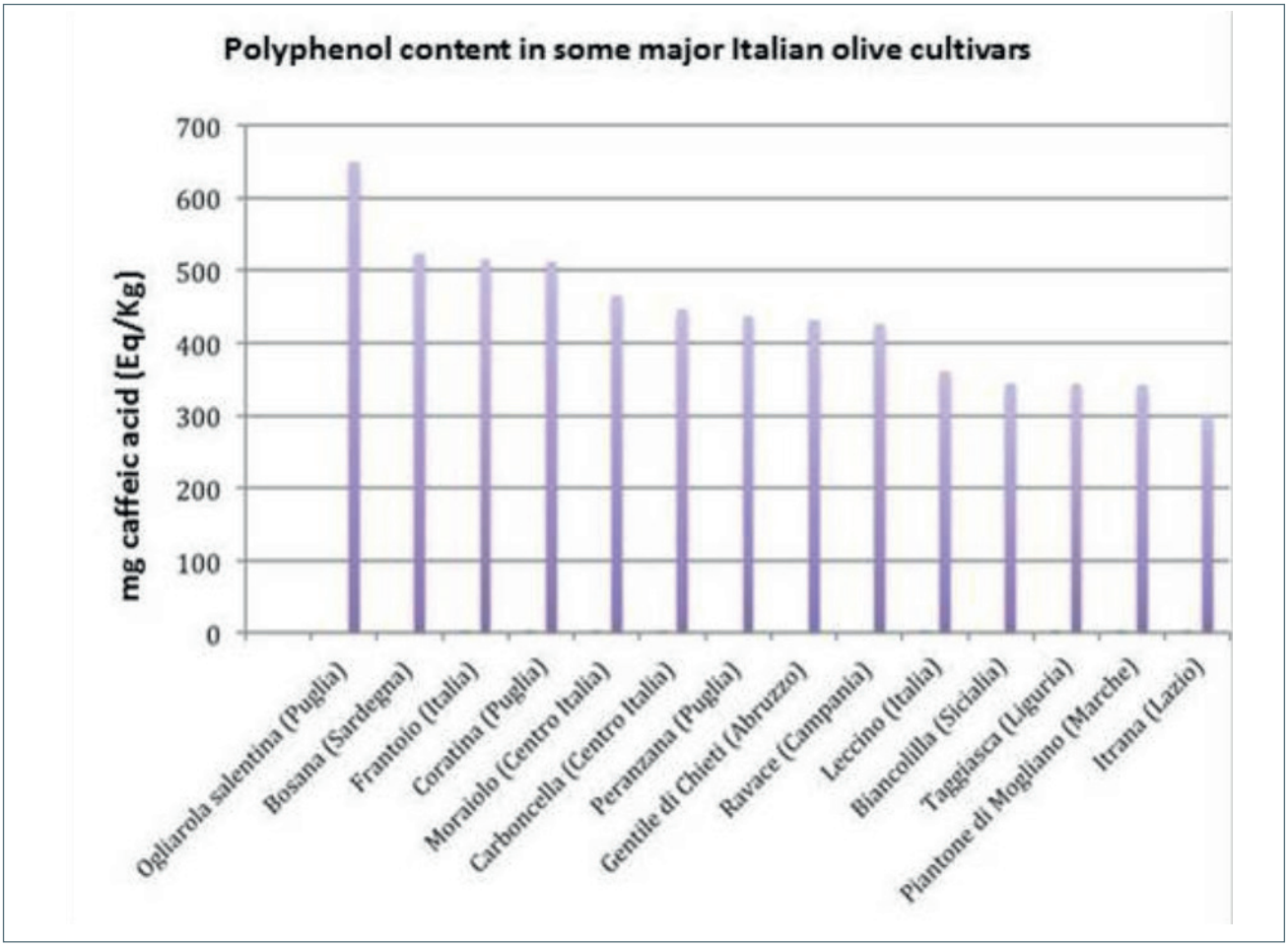

Figure 7. Polyphenol content in some major Italian olive cultivars.

diet and risk of cardiovascular disease [17]. Subsequently, the *Three-City Study*, carried out on 7,000 subjects and published in 2009, suggested the existence of a significant correlation between olive oil consumption and reduced risk of age-associated cognitive impairment [18]. A recent analysis of the scientific literature related to clinical trials and population studies has confirmed these ideas, leading to conclude that the MD, particularly when supplemented with olive leaf extract-based nutraceuticals, provide consistent and significant protection against the risk of major chronic degenerative diseases including cardiovascular disease, cancer, T2DM and neurodegenerative diseases [15 19 20]. Table I shows the main beneficial effects associated with the consumption of olive oil.

Olive oil and olive leaf extracts exert their beneficial effects against CVD by different molecular mechanisms. The reduction of the risk factors of CVD is due not only to the high levels of monounsaturated fatty acids but also to other compounds found both in the olive oil and in olive leaf-extracts. Monounsaturated fatty acids modify the lipid profile by reducing both total and LDL-cholesterol, while leaving unmodified or increasing HDL-cholesterol; they also decrease LDL oxidation, a key modification in atherosclerotic plaque formation and growth. The high consumption of monounsaturated fatty acids and the reduced consumption of saturated fatty acids, typical of the MD together with other features, including

Table I. Beneficial properties of olive oil (evidence from nutritional intervention studies in different populations).

Reduction of LDL-cholesterol and increase of the ratio total cholesterol /HDL-cholesterol
Reduction of non-alcoholic fatty liver disease
Reduction of the oxidation of LDL-cholesterol
Improvement of glucose metabolism, reducing blood glucose and insulin, and insulin resistance
Improvement of endothelial function
Antithrombotic effect with reduction of some thrombogenic factors

the intake of polyphenol-enriched olive leaf extracts, also result in increased protection against the onset of obesity, a major risk factor for T2DM and the metabolic syndrome. It has been shown that a typical MD, in which 50% of the energy is provided by carbohydrates and 35% by lipids (mainly monounsaturated), results in a significant reduction of glycated hemoglobin and improved glycemic control respect to a standard diet [21]. These effects also appear associated with the amount of olive oil polyphenols taken up (see below).

In general, the beneficial effects of olive oil have been consistently attributed to the content in polyphenols, due to the antioxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-viral, anti-atherogenic, hypoglycemic, liver- heart- and neuro-protective power of the latter [22-26]. In addition to the effects on lipid and glycemic parameters, several studies confirm a reduction in blood pressure in people who follow a Mediterranean-style diet rich in monounsaturated fatty acids and olive oil [27]. Finally, in recent years, in addition to the beneficial effects against the risk factors for CVD and T2DM reported above, several studies deal with a protection by olive oil and olive leaf extracts also against thrombosis-related factors (hemostasis primary, secondary, platelet aggregation, fibrinolysis) that contribute to the onset of CVD [28]. Beneficial effects of olive oil and polyphenols-enriched olive leaf extracts against neoplastic diseases have also been reported in various studies carried out mainly in animal and cell models [19 29].

Metabolic effects of oleuropein

Oleuropein aglycone (OLE), together with its main metabolite, hydroxytyrosol, is considered the main responsible for many nutraceutical properties of olive oil and olive leaf extracts. Recent studies on OLE have provided a more detailed scientific basis for the reported anti-aging effects of the MD and the beneficial properties of olive oil, particularly against T2DM and other conditions associated with the metabolic syndrome. The beneficial effect of OLE against T2DM is suggested by a number of experiments on animal models and by clinical trials on human subjects, even though the latter are still limited also for what their number and the number of enrolled people are concerned. The scientific literature supports the beneficial properties of OLE and OLE-enriched olive leaf extracts in animal and cell models of T2DM. In particular, it has been reported that olive polyphenols (i.) prevent amylin tendency to aggregate into amyloid fibrils whose pancreatic deposits are considered among the main causes of the sufferance and functional impairment of insulin-secreting cells in T2DM (30); (ii.) decrease blood glucose and cholesterol levels by repairing the oxidative damage in diabetic murine [31 32] and rabbit [33] models; (iii.) reduce starch digestion and intestinal absorption of dietary carbohydrates in murine models of diabetes [34]; (iv.) improve oral glucose tolerance in rats at carbohydrate- and lipid-rich diet [35]; (vi.) modify the expression, among others, of genes involved in lipogenesis and insulin resistance, in mice fed with high-fat diet [36]. Olive polyphenols also appear to prevent the onset of T2DM by increasing the tolerance to oral glucose and by mitigating high-fat diet-induced fatty liver and obesity in murine models [34 37-39].

Clinical studies have also been carried out in human subjects whose diet contained controlled amounts of olive oil. From these studies it emerged that olive oil polyphenols improve glucose homeostasis and reduce glycated hemoglobin and fasting insulin levels [40]. Very recently, a study carried out by Italian researchers has reported that the intake of polyphenol-rich olive oil during lunch by normal subjects reduces significantly the peak of postprandial glycaemia [41]. The study confirms a preceding one on the effects of OLE on glucose metabolism showing a sharp reduction of both postprandial blood glucose and of glycated hemoglobin in subjects administered with OLE [42]. Finally, as reported above, a clinical trial was recently carried out in New Zealand on a group of middle-aged overweight individuals at risk for development of the metabolic syndrome treated for 12 weeks with an olive leaf extract enriched in OLE and, in a minor amount, of oleocanthal [36]. At the end of the treatment, the subjects showed a significant improvement in insulin sensitivity and insulin-secreting pancreatic cell function, suggesting a significant anti-diabetic effect [36]. Even though carried out on small cohorts of subjects, these results suggest that olive polyphenols, particularly OLE, possess significant anti-diabetic power, particularly against T2DM, and agree with *in vitro* results on the effect of OLE against amylin aggregation [30].

Another disease related to insulin resistance and the metabolic syndrome is non-alcoholic fatty liver disease (NAFLD) and the ensuing nonalcoholic steatohepatitis (NASH). Studies on cell and animal models report that OLE can counteract these states in several ways. These include (i.) an anti-lipidemic action [43]; (ii.) the protection of cultured cells against hepatocellular steatosis induced by free fatty acids [44]; (iii.) the protection against liver damage in CCl_4-treated mice [45]; (iv.) the prevention of the occurrence of spontaneous NASH in a mouse model [46]; (v.) the prevention of the progression of NASH toward fibrosis in high-fat diet mice [47]; (vi.) the dose-dependent suppression of the intracellular accumulation of triglycerides during adipocyte differentiation [38]; (vii.) the reversal of weight increase of the liver and the decrease of blood lipid levels in high-fat diet mice by interfering with signaling

pathways involved in lipogenesis and in the onset of fatty liver [29 31 38 48]. The positive effects of OLE on NASH have been shown in a recent study carried out in mice fed with a normo-caloric diet, with high-fat diet or with high-fat diet supplemented with 3% OLE for a further eight weeks [23]. These studies have not been replicated in human subjects; accordingly, the efficacy of OLE and other olive polyphenols against these disease in humans is not adequately supported and remains unproven.

Olive polyphenols could be protective against Alzheimer's disease: the diabetes-AD link

Evidences from epidemiological, cell biology and animal models suggest that pre-diabetes and diabetes increase the risk of dementia and that the risk to develop AD is increased by 2-3-fold in patients with diabetes, notably T2DM [49]; in particular, recent research has highlighted the importance of brain insulin signaling and that insulin-resistance may lead to AD [50]. Accordingly, a close relation between diabetes and dementia [51], particularly AD, has been proposed, possibly through protection against alterations in mitochondrial function/biogenesis and in autophagy [52]. Even though the relation between AD and diabetes has been questioned very recently [53], many data suggest that impairment of brain insulin signaling is at the core of the neurodegeneration cascade in late onset AD, leading some authors to define some AD symptoms as "brain-type diabetes" or "type 3 diabetes" [54 55]. Therefore, it is not surprising that recent research has reported a significant protection by OLE not only against T2DM but also against brain neurodegeneration and the ensuing behavioral and memory impairment; the latter data have been reported in a number of studies carried out on TgCRND8, a mouse model of Abeta deposition [56]. However, these studies have not been replicated in human subjects; accordingly, the efficacy of OLE and other olive polyphenols against neurodegeneration in humans has not been proven yet.

OLE and oleocanthal were previously shown to modify favorably the tendency of the Abeta peptide and tau protein to aggregate in vitro into cytotoxic amyloid assemblies [57 58]; they were also shown to protect transgenic animal models against Abeta aggregation and aggregate toxicity [44 59] in several ways, including a strong activation of autophagy [44], a protective response known to be deficient in brain dementia [60-63]. Hydroxytyrosol, the main product of OLE metabolism has also been shown to be protective not only due to its high anti-oxidant power but also by sharing most of the above mentioned effects of OLE both in cell and in animal models (reviewed in [64]), particularly against neurodegeneration (reviewed in [65]).

Molecular determinants of the beneficial effects of olive polyphenols

The effects of OLE and other olive polyphenols have also been studied at the molecular level in cell and animal models as well as in human subjects. The reported molecular modifications following administration of olive oil, olive extracts or pure polyphenols include (i.) the down-regulation of the expression of pro-atherogenic genes in a clinical trial with healthy volunteers upon assumption of olive polyphenols in the context of a traditional MD [66]; (ii.) the prevention of cytokine-mediated inflammation and oxidative damage [43 48]; (iii.) the increase under fasting conditions of the levels of signaling molecules such as IL-6, IGFBP-1 and IGFBP-2 [36]. The anti-obesity and anti-steatosis effects were associated with increased metabolic utilization of lipids and energy expenditure and with the modulation of glucose homeostasis (see above); they also appear to depend on the down-regulation of the expression of genes involved in the differentiation of adipocytes [38] and in Wnt10b inhibition as well as on the increased expression of genes involved in thermogenesis [39] and mitochondrial biogenesis in visceral adipose tissue [40]. Finally, the molecular effects underlying the anti-neurodegeneration power of olive oil polyphenols include, in addition to autophagy activation, increased amyloid-β clearance from the brain by oleocanthal [67] and reduction of Aβ production by OLE through the promotion of the non-amyloidogenic pathway following increased α-secretase cleavage of the amyloid precursor protein [68].

The reported effects of OLE are similar to those produced by other natural polyphenols found in typical foods of the MD and the Asian diet [20]. Often these effects are the result of modifications of the expression of genes involved in epigenome modulation, as recently shown in the case of OLE and other polyphenols, resulting in protection against numerous cancers [69] and neurodegenerative disorders [69-71]. Figure 8 summarizes the most referenced healthy effects of OLE and its metabolites reported in animal models and/or in humans.

Bioavailability of dietary polyphenols

It is commonly believed that OLE and other natural polyphenols are, in general, poorly bioavailable both because of their reduced intestinal absorption and of their rapid biotransformation which helps their urinary excretion. Nevertheless, recent studies conducted in rats and in humans have shown that these compounds are indeed absorbed in reduced, yet appreciable, amounts from the intestine and rapidly distributed throughout the body, including the brain [72-74]. The administration of polyphenols-enriched nutraceuticals is hindered by the lack of in depth studies about the effective dose to be administered daily in humans to

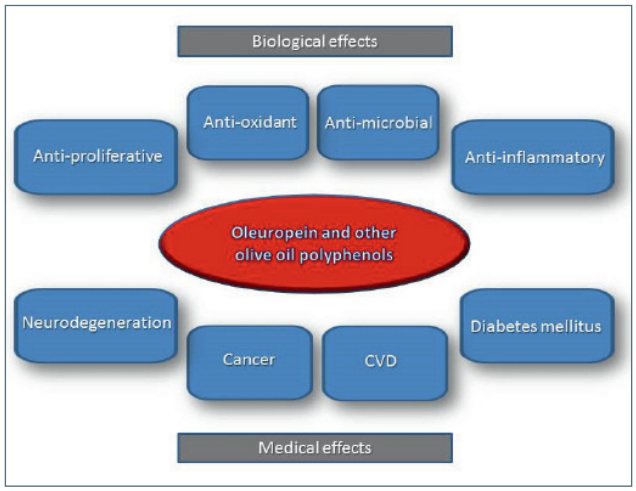

Figure 8. A schematic view of the healthy effects of oleuropein.

get acute effects. Actually, it appears that the amount of OLE and other polyphenols in food is not adequate to ensure the intake, with a common diet, of doses that can produce short-term acute effects. Yet, clinical and experimental evidence suggest that a continuous intake of foods containing low concentrations of these molecules can be effective in the long term, representing a continuous low intensity stimulus of the cellular defenses against T2DM, CVD, the metabolic syndrome and aging-associated neurodegeneration. Therefore, following a nutritional style conformed to the MD appears to provide a useful protection against the risk of the metabolic syndrome, particularly T2DM, whereas more rapid and acute effects against the latter, seem to require a significantly higher daily intake of plant, notably olive, polyphenols.

CONCLUSIONS

The results of experimental studies carried out in cultured cells and model animals as well as the efficacy evidence in humans, confirmed by recent population studies and clinical trials [18 21 27 36 41 42 66 72], provide consistent support to the use of OLE in dysmetabolic states of carbohydrates and lipids as well as, possibly, in neurodegeneration. However, these data must still be confirmed by larger population studies, mainly for what OLE protection against aging-associated neurodegeneration is concerned. Ongoing studies, both experimental, clinical and observational, on the metabolic effects of olive polyphenols will further confirm or resize the role of these molecules, particularly OLE, as diet supplements or even nutraceuticals useful for the prevention of aging- and lifestyle-related degenerative conditions including T2DM, the metabolic syndrome and aging-associated neurodegeneration.

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Review

Aging and aging theories

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Several theories have sought to explain aging, here precisely defined as "increasing mortality with increasing chronological age in populations in the wild". They all fall within one of two opposite and incompatible paradigms. For the first ("old paradigm"), aging is the result of degenerative phenomena that natural selection cannot counteract completely, due to insufficient strength or opposing selective pressures. For the second ("new paradigm"), aging is favoured by natural selection in terms of supra-individual selection: it belongs to a broader category of phenomena, on the whole defined as "phenoptosis", which are explicable only in terms of supra-individual selection. For the new paradigm, aging is a specific function that is genetically determined and regulated, with its own physiology, pathology and phylogeny. This paper describes the theoretical arguments and the empirical evidence that support or are in contrast with each of the two paradigms. Subsequently, on the basis of an imposing and authoritative amount of research, aging mechanisms at the cellular and organismal levels are described. The clear existence of such mechanisms is indispensable proof to support the new paradigm and is in complete and unsolvable contrast with the old paradigm.

Key words: Aging, Phenoptosis, Supra-individual selection, Telomere, Subtelomere, Cell senescence, Cancer

INTRODUCTION

Definition of aging

Aging is here defined as "increasing mortality with increasing chronological age in populations in the wild", or "IMICAW" [1], a definition that is analogous to others such as "actuarial senescence" [2] and "progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age" [3] with the essential difference that these do not have the condition "in the wild".

It is essential that this condition is present and explicit because its absence may lead to false conclusions. In fact, let us consider a species that shows no mortality increase in the wild, but under protected conditions, e.g., in captivity, may reach ages, which are non-existent in nature, where there is evidence of an age-related increasing mortality (e.g., see below the case of the spider *F. pyramitela*). For the first definition, this species does not age; for the other two definitions, the species may be considered as subject to aging. However, a death rate increase that is not present in the wild and is shown, only under protected conditions, at ages which are non-existent in the wild cannot be subject to natural selection. So, its causes cannot be an explanation for the increase in mortality shown by other species under natural conditions.

It is also important to have full awareness that aging, as described in the first definition, exists and that this is well documented from a long time [2 4-9], for our species too [10] (Fig. 1). The existence of the phenomenon has been minimized and deemed insignificant ("there is scant evidence that senescence contributes significantly to mortality in the wild" [3], "senescence-associated increases in age-related mortality… even where they are observed, they contribute only to a relatively small fraction of deaths within the population" [11]), but Ricklefs highlighted that senescence reduces average life span

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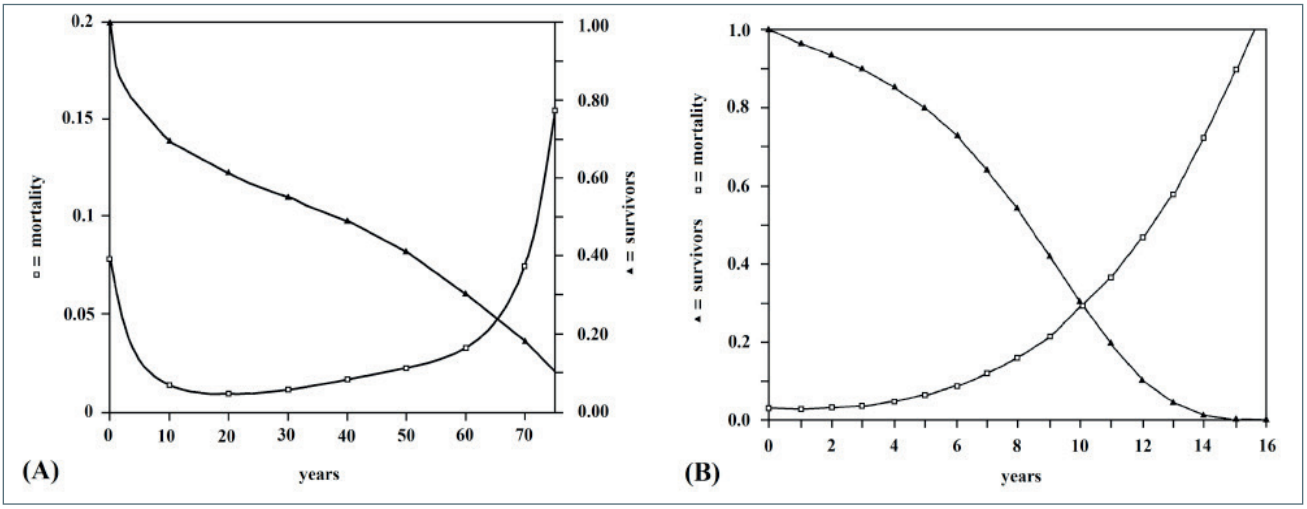

Figure 1. Some examples of aging. A: *Homo sapiens* (Ache population, data from Hill, Hurtado, 1996 [10]); B: *Pantera leo* (data from Ricklefs, 1998 [9]); for both species, observations in the wild.

up to "almost 80%" [9] and, later, a meta-analysis highlighted the evidence of aging in 175 animal species on the basis of 340 separate studies [12].

Classification of aging theories

Among the many theories that try to describe the causes of aging [13-16], a first possible distinction is between non-evolutionary and evolutionary theories. The theories of the first group are formulated without any consideration of the natural selection as possible factor that somehow affects aging. Within this group there are almost all of the oldest hypotheses, including those explaining aging as a result of progressive wear and tear. In the second group, there are theories that in various ways try to reconcile their explanations of aging with the mechanisms of natural selection.

A more interesting distinction is between two different and opposing interpretations:

1) aging is a non-programmed phenomenon; it is a set of degenerative phenomena that natural selection cannot contrast completely due to insufficient strength or opposing selective pressures;
2) aging is a programmed phenomenon; it is caused by mechanisms genetically determined and programmed that, despite being harmful to the individual, are in some way advantageous in terms of supra-individual natural selection.

As the contrast between the two interpretations is strong and complete and does not appear solvable by some form of compromise, the two interpretations have the value of opposite paradigms, in the sense of the term defined by Kuhn [17].

All non-evolutionary theories, and a large part of the evolutionary theories, refer to the first interpretation, defined as "old paradigm". It includes a significant number of hypotheses according to which aging is caused by the progressive accumulation of damage of various types and the consequent fitness impairment. In the older theories, the phenomenon is conceived without any consideration of the evolutionary mechanisms, i.e., with the implicit assumption that natural selection is irrelevant for this phenomenon [18-23]. Some less old theories consider natural selection and propose that the damaging mechanisms are poorly contrasted by selection, (i) as few individuals survive at older ages, (ii) for the constraints imposed by genes with pleiotropic effects, (iii) for the limits caused by other physiological needs [24-43]. For all the hypotheses of the old paradigm, aging: (i) is not favoured by natural selection, and so (ii) cannot have specific mechanisms genetically determined and regulated that determine it. Furthermore, as aging is seen as a set of degenerative processes, the term "aging" must be considered as a useful word to summarize the overall effects of heterogeneous phenomena: aging as a distinct entity does not exist. According to this paradigm, which is currently dominant: (i) in the present International Classification of Diseases [44 45], there is no code for aging, (ii) aging as a distinct cause of death is excluded and, for the international official statistics of the World Health Organization, aging as a distinct cause of death is left out [46].

Only some of the evolutionary aging theories refer to the second interpretation, defined as the "new paradigm". They interpret aging as a physiological phenomenon, determined and regulated by specific genetically programmed mechanisms, which are favoured by natural selection as advantageous in terms of supra-individual selection despite the disadvantages caused by them

on the individuals [1 47-68]. It is intrinsic to this conception that the aging mechanisms must have (i) a physiology, (ii) a pathology, and (iii) a phylogeny.

Some basic concepts

Some essential premises are necessary for the subsequent discussion.

A) Subjects of aging theories

It is essential to make a distinction about the specific topics of aging theories. In fact, a first subject is the explanation of the "why" of aging in evolutionary terms and another subject is the "how" of aging. For the theories that attempt to explain aging without considering evolutionary mechanisms, this distinction does not exist, and the "why" and the "how" are the same thing. Even for some of the theories that try to take into account the mechanisms of evolution but attribute aging to an insufficient selection against damaging factors, the distinction between the "why" and the "how" is weak or non-existent. On the contrary, for other evolutionary theories the discussion about the "why" is clearly distinct from the discussion about the "how".

B) Various descriptions of natural selection

In its most famous and popular simplification, natural selection is "the survival of the fittest" of Spencer [69], an expression adopted later by the same Darwin ("Natural Selection or the Survival of the Fittest" [70]), i.e., in modern terms, the preferential spreading of the genes of individuals who are fittest to survive and reproduce.
This may be expressed by a simple formula that tells us the condition for which a gene (C) is favoured by natural selection:

$$S \times P > 0, \quad (1)$$

where: S = advantage caused by the expression of C; P = reproductive value of the individual at the age when C is expressed.
In a more general conception, natural selection operates in terms of kin selection [71-74]. It is necessary to consider the inclusive fitness of a gene (C) whose action has effects not only on the individuals I_1, where C exists, but also in individuals I_2, I_3,... I_n, which are related with I_1 and for which there is a probability that C is in the genome equal to the coefficient of kinship (r) between I_x and I_1. Therefore, C will be favoured by natural selection when:

$$\sum_{x=1}^{n} (S_x \times P_x \times r_x) > 0 \quad (2)$$

Clearly, when n = 1, as $r_1 = 1$, formula (2) becomes formula (1), and so individual selection is only a particular case of kin selection.
Now, as already discussed in another paper [75], if we consider a species:

- subdivided into monoclonal demes and subjected to catastrophic events that cause a disadvantage S for every individual;
- in which, by action of a gene (C), among the n individuals with C, some (n_d) sacrifice themselves and die ($S_d = -1$) while the survivors (n_s) have an advantage S_s;
- for the sake of simplicity, the reproductive value is assumed to be constant at any age ($P_x = 1$).

by considering that in a monoclonal deme $r_x = 1$, the formula (2) becomes:

$$\sum_{x=1}^{n_d} S_d + \sum_{x=1}^{n_s} S_s > S \times n, \text{ that is: } n_d \times S_d + n_s \times S_s > S \times n \quad (3)$$

Moreover, if we suppose that in the deme there are several clones (1, 2,..., z) and C exists in all the individuals of the first clone, the probability that C is in the individuals of a clone x is equal to the coefficient of kinship between the individuals of clone x and those of clone 1 (r_x), and C will be favoured by natural selection if:

$$(n_{1,d} \times S_d + n_{1,s} \times S_s) + (n_{2,d} \times r_2 \times S_d + n_{2,s} \times r_2 \times S_s) \ldots + (n_{z,d} \times r_z \times S_d + n_{z,s} \times r_z \times S_s) > S \quad (4)$$

where, in a clone x: $n_{x,d}$ = the individuals that sacrifice themselves; $n_{x,s}$ = the survivors.

By considering these particular conditions, and certainly other possible cases, the inclusive fitness formula is transformed into equations that describe how C could be favoured in terms that are definable as group selection.
As a further significant example, the social organization (eusociality) of haplodiploid species such as ants, bees and wasps was described for many years as a result of mechanisms of kin selection [74 76], but later, together with the eusociality of other non-haplodiploid species such as termites, bathyergid mole rats etc., "the standard natural selection theory in the context of precise models of population structure", which includes "multilevel selection", was considered a better and more fruitful explanation [77]. Also in this case natural selection is always the same phenomenon but is studied in different conditions and through different mathematical models.
This shows that individual selection, kin selection and at least certain types of group selection are always natural selection but under different conditions or with

a different descriptive approach. Moreover, this means that some old arguments against group selection as a possible valid form of natural selection [78-80] should be reconsidered. The key concept is that if we exclude individual selection, all the other descriptions of natural selection can be described by the comprehensive term "supra-individual selection": the substantial difference between these two categories of natural selection is that individual selection cannot justify a gene that is detrimental to the individual, while, in contrast, supra-individual selection may favour, under particular conditions, genes that are harmful or even fatal for the individual.

C) The concept of "phenoptosis"

Apart from the cases of eusociality, these theoretical considerations have a sure confirmation in a wide range of phenomena in which an individual sacrifices himself, or a closely related individual, through the direct or indirect effect of genes favoured by natural selection, in terms of supra-individual selection. These phenomena, although very common and well known for a long time (see the chapters: "Rapid Senescence and Sudden Death" and "Gradual Senescence with Definite Lifespan" in Finch's 1990 textbook [8]), until a few years ago did not have a general term that defined them. Skulachev proposed this needed definition at the end of the nineties: "Phenoptosis [is] the programmed death of an individual" [56 57], and afterwards this concept has been extended to the sacrifice of related individuals ("Phenoptosis is the death of an individual caused by its own actions or by actions of close relatives… and not caused primarily by accidents or diseases or external factors, which is determined, regulated or influenced by genes favoured by natural selection" [54]).

Aging, seen as an event that is favoured and determined by natural selection, falls into the category of phenoptotic phenomena and was indeed defined by the same Skulachev as "slow phenoptosis" [81 82].

D) Non-universality of aging

A widespread belief is that aging, as before precisely defined (age-related mortality increase in the wild), is a phenomenon shown by all living species with few exceptions. In contrast, the natural observation shows us that aging is shown only by a small number of species, ours included, although these species are among those most familiar to us. A recent work has shown among the numberless species an incredible variety of life tables or age patterns of mortality [83], in particular species with no age-related mortality increase (Fig. 2).

In fact, some species show "no observable increase in age-specific mortality rate or decrease in reproduction rate after sexual maturity; and… no observable age-related decline in physiological capacity or disease resistance" [84] (e.g., rockfish, sturgeon, turtles, bivalves and possibly lobsters [84]). They have been defined species "with negligible senescence" [8]. Indeed, individuals of these species do not grow old but this is difficult to admit for some current theories (see below): the aforesaid expression is a prudent way of saying that they could also grow old but the pace is so slow as to be undetectable.

In particular species, there is even an age-related decrease in mortality. These are species whose death rate would be constant at all ages except that the age-related increase in body size causes less vulnerability to predation and then reduces mortality. The definition "negative senescence" has been coined for them [85], but, perhaps more correctly, we should consider these species as a particular type of species with "negligible senescence".

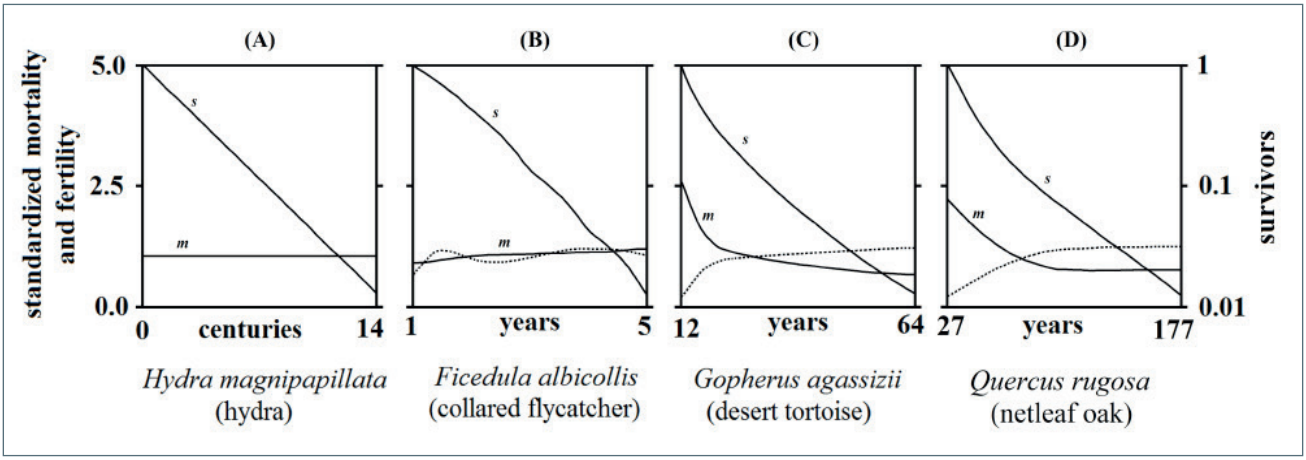

Figure 2. Some examples of life tables of non-aging species (partial and redrawn Figure 1 of Jones, Scheuerlein, Salguero-Gómez, et al., 2014 [83]). Solid lines indicate standardized mortality (*m*) and survivorship (*s*), the dotted lines the standardized fertility. (A) and (B) are cases of "negligible senescence", (C) and (D) are examples of "negative senescence". In (A), mortality and fertility lines overlap.

Other species do not age, but, at the time of reproduction, their individuals suddenly undergo rapid degenerative processes that cause imminent death (e.g., many Anguilliformes and Salmoniformes, some rodents and dasyurid marsupials, many plants, in particular monocarpic angiosperms [8]). This type of phenomena, defined by Finch as "sudden senescence" [8] is quite distinct by aging as before defined.

Many species are congenitally incapable of being able to live more than a short time. "Aphagy from defective mouthparts or digestive organs is very common during the adult phases of insects (Weismann, 1889b; Metchnikoff, 1915; Norris, 1934; Brues, 1946; Wigglesworth, 1972; Dunlap-Pianka et al., 1977) and is *the* limiting factor in the adult lifespan of many short-lived species" [8].

Other species, including many insects and spiders, in the wild have high mortality and show no age-related increase in mortality during their short lives (e.g., under natural conditions, the lifespan of *Frontinella pyramitela* ("bowl and doily" spider) is less than 30 days and shows no age-related increase in mortality). However, under laboratory conditions, at ages that are non-existent in the wilds, this spider shows an age-related increase in mortality that is strongly conditioned by the amounts of available food [86] (Fig. 3). As this mortality increase happens only under artificial conditions, it is outside the definition of aging.

It is possible to indicate other particular cases but, for the sake of brevity, we refer to the cited work [83]. However, a consideration is necessary and due. If we weigh the enormous number of species that do not age, and consider that aging occurs in a minority of species, we must agree as a matter of fact that aging is not an inevitable and almost universal condition but, on the contrary, a peculiar condition of a limited number of species.

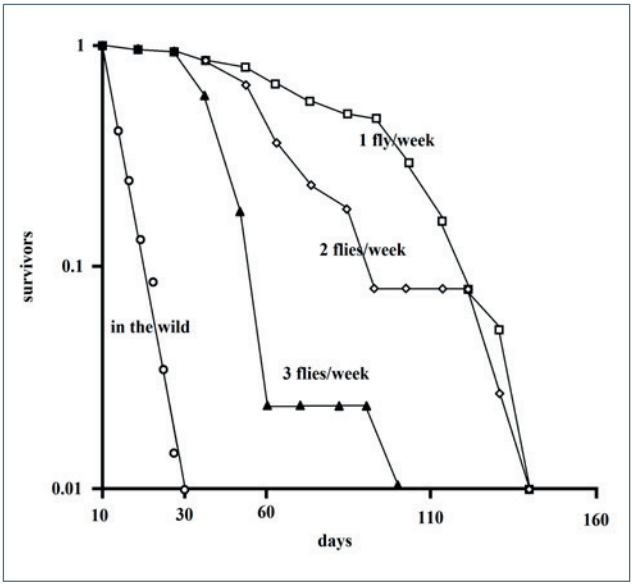

Figure 3. Survival of *Frontinella pyramitela* in the wild (circles) and in laboratory in different feeding conditions: 1 fly/week (squares); 2 flies/week (rhombs); 3 flies/week (triangles); data from Austad, 1989 [86].

THE "WHY" OF AGING

Non-programmed aging theories

The "classical" evolutionary theories that try to explain aging are three and are all within the old paradigm. The first, *mutation accumulation hypothesis*, explains aging as the combined effect of many harmful genes that act later in life and are insufficiently removed by natural selection [24 26 27 32]. A simple theoretical argument against this hypothesis has been proposed for a long time [1 51] and proposed again [16 87], but no one has attempted to invalidate it.

In short, if we have a gene (C) that is harmful and causes a disadvantage *s*, with a neutral allele (C') and a mutation frequency from C' to C equal to *v*, it is possible to obtain the equilibrium frequency between mutations C' -> C and their elimination by natural selection. From this equilibrium frequency we calculate the frequency of the phenotypic expression of the gene (P_e) both in the case that C is recessive:

$$P_e = v/s \quad (1)$$

and in the case that C is dominant:

$$P_e \approx v/s \quad (2)$$

The details of this calculation are explained elsewhere [87]. Now, let us hypothesize genes that are harmful, by a value *s*, at time t and with no effect on preceding ages. As these genes ("t-genes") are harmful only for the survivors at time t (Y_t), natural selection contrast them in function of $s \times Y_t$ and the equations (1) and (2) become:

$$P_e \approx v/(s \times Y_t) \quad (3)$$

In a population with a death rate (λ) that is constant at any age, namely, a non-aging population, the life table is obtained from the simple equation:

$$Y_{t+1} = Y_t \times (1 - \lambda) \quad (4)$$

By supposing n t-genes that act at time t, as many t-genes that act at t+1, and so on, and that, for the sake of simplicity, the harm caused by each of these has always the value *s*, the survivors at t+1 will be:

$$Y_{t+1} = Y_t \times (1 - \lambda - n \times s \times P_e) \approx Y_t \times (1 - \lambda - n \times v / Y_t) \quad (5)$$

This equation (5) is independent from the value of *s* and, as the value of *v* is small, the decrease in Y from t to t+1 will be notable only with small values of Y_t.

Curve C in Figure 4 shows the effects of a great number of t-genes (n = 1000) on a life table with a constant death rate (curve B). Curve C is completely different from that of a real population (curve A), which, in the first ages, has the same mortality as the other two curves, but afterwards shows a progressive age-related increase in mortality.

The second of the "classical" theories, the *antagonistic pleiotropy hypothesis* [25 33], postulates the existence of many genes that are harmful at older ages but advantageous at earlier ages. Therefore, natural selection contrasts them only in part, and organisms grow old.

The third theory, the *disposable soma hypothesis* [29 30], postulates the existence of mechanisms that are useful and advantageous at the young or adult stage but harmful at later ages. The body must economize resources, which are not well defined by the theory, and so natural selection, by these mechanisms, operates a compromise in the allocation of resources, which must be divided between reproduction or other physiological needs and the preservation of soma integrity that would allow for greater longevity.

These two theories are not vulnerable to the theoretical argument presented earlier. However, all the three classical hypotheses, together with those that explain aging as caused by the accumulation of harmful effects, do not explain the huge variability of aging rates in the comparison among species and do not justify in any way the existence of species in which the death rate is constant at any age. Perhaps *ad hoc* hypotheses could try to explain: (i) why the mechanisms proposed act to varying degrees depending on the species, (ii) why they do not act at all in some species. However, a theory cannot be considered plausible if it is built on postulates and *ad hoc* assumptions.

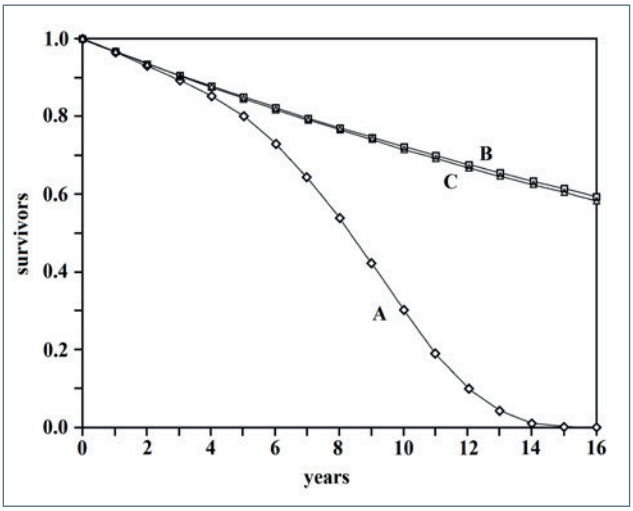

Figure 4. Hypothetical effects of a great number of t-genes on a life table. Curve A (rhombs): life table of *Panthera leo* in the wild; the death rate is described by Weibull's equation ($m_t = m_0 + \alpha t^{\beta}$) with the values $m_0 = .032$; $\alpha = .000252$; $\beta = 3$ given by Ricklefs, 1998 [9]. Curve B (squares): a life table with constant mortality equal to m_0 of *Panthera leo*. Curve C (triangles): the curve B plus the effects of many t-genes (n = 1000; *v* = .000001).

There is also another strong argument against any hypothesis of aging interpreted as non-programmed phenomenon.

In the formulation of the first theory that hypothesized aging as planned and favoured by natural selection, it was proposed that the supra-individual advantage of aging originated from the reduction of the mean duration of life (ML). It followed from this that, in case of major extrinsic or environmental mortality, the hypothesized advantage caused by ML reduction was lower and therefore the proportion of deaths due to aging could be reduced. Therefore, in a paradoxical way, the theory stated that extrinsic mortality and ML reduction caused by aging had an inverse relationship [1 51]. Subsequently, it was observed that this prediction should be valid for all theories that propose aging phenomenon as planned and favoured by natural selection [88]. In particular: "… senescent mortality tends to complement background mortality. Both contribute to the population turnover rate, and thus to evolvability… [the] relationship between background death rate and evolved senescence is characteristic of adaptive theories of aging. A high background death rate leads to a longer evolved life span. This contrasts with classical theories, in which a high background death rate leads to a shorter evolved life span" [68].

The three classic hypotheses, and, implicitly, also the non-evolutionary theories of aging, formulate the opposite prediction. According to these hypotheses, since aging is countered, though insufficiently, by natural selection, the increase in extrinsic mortality weakens natural selection, and therefore aging should be accelerated. So, a direct relationship between mortality and extrinsic aging rates is predicted: "The principal determinant in the evolution of longevity is predicted to be the level of extrinsic mortality. If this level is high, life expectancy in the wild is short, the force of selection attenuates fast, deleterious gene effects accumulate at earlier ages, and there is little selection for a high level of somatic maintenance. Consequently, the organism is predicted to be short lived even when studied in a protected environment. Conversely, if the level of extrinsic mortality is low, selection is predicted to postpone deleterious

gene effects and to direct greater investment in building and maintaining a durable soma" [3].
However, in 1998, Ricklefs' data on populations studied in the wild showed that the inverse relationship predicted by the hypothesis of aging as a programmed phenomenon was true [9] (Fig. 5).
This plain contradiction between the empirical data and the predictions of the three classical theories was underlined by Ricklefs [9] and was subsequently deepened [88]. However, for this contradiction, there remains no satisfactory explanation that might be compatible with the aforementioned classical theories and with non-adaptive theories of aging.

Programmed aging theories

Alfred Russel Wallace, who co-authored the first paper on the theory of evolution through natural selection with Charles Darwin, was also the first who, in 1865-1870, proposed that aging was programmed because individuals who die as a consequence of aging do not compete with their offspring [65 89]. Likewise, August Weissmann, in 1889, hinted that aging was somehow favoured by natural selection because the death of old individuals frees space for the younger generations and so for the spread of new genes [47 50], but a few years later, he dismissed this idea [48 50].
In 1961, a botanist proposed again the argument that senescence accelerates generation turnover and so "… in plants senescence is a catalyst for evolutionary adaptability" [49].
In 1988, after an anticipation in a non-peer reviewed book [51], a theory was proposed that explained aging as adaptive in spatially structured populations and in terms of kin selection because it accelerated evolution [1]. This hypothesis, which was later reaffirmed [52 53 55 88], starts from the following consideration.

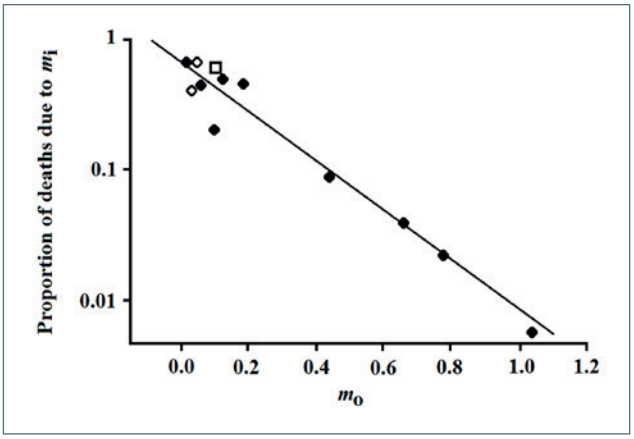

Figure 5. Inverse relationship between m_0 and the proportion of deaths due to m_i. Solid diamonds refer to bird species, open diamonds to mammal species, open square to *Homo sapiens*; ordinates are in logarithmic scale. Data for mammals and birds are from Ricklefs, 1998 [9], Table II; data for *H. sapiens* are from Hill, Hurtado, 1996 [10]. Without the data of our species, the linear regression has the following values: r = -.758708, t = -3.494043, p < 0.01 (from Libertini, 2008 [88]).

The spread within a species of a favourable gene (C) with an advantage *s*, is a function of both *s* and the speed of generation turnover, which is inversely proportional to the mean duration of life (*ML*) of the individuals. If *s* is multiplied for x or if *ML* is divided by x, we will have exactly the same effect on the spreading of C (Fig. 6).
So, a shorter ML has the great advantage of a higher spreading diffusion for all favourable genes (and also a quicker elimination of all unfavourable alleles), but also entails the disadvantages that result from the shorter ML (which are increased by a greater body mass and a greater duration of the physical and neurological maturation periods). However, it was noted that, in populations divided into small groups of related to each other individuals and in condition of demographic saturation (i.e., k-selection [90]), the advantage would overcome the disadvantages and a hypothetical gene (C) determining a reduced *ML* ($ML_C < 1$) would be favoured by selection against a neutral allele C' (with $ML_{C'} = 1$) if:

$$r \times S \times (1/ML_C - 1) > S' \quad (6)$$

where: *r* = coefficient of relationship among the individuals of the group; *S* = summation of the advantages of all the favourable genes that are spreading; *S'* = summation of the disadvantages for the individual caused by a reduced *ML*.
In the following years, some theories also proposed that aging was favoured by natural selection in spatially structured populations [63 67 68]. In fact, these new contributions proposed again the same advantage for aging that resulted from a faster gene spreading but by using more sophisticated models of population genetics.
However, the first and the new theories predicted that in the case of populations not divided into groups, or those with unlimited dispersal, the aging genes were not favoured by natural selection (e.g.: "In a freely mixing population with global dispersal, evolution selects for individuals with ever-increasing life span" [63]).
Another theory, in 2009, explained aging as a defence against the spread of infective diseases, analogous to the Red Queen hypothesis on the advantages of sexual reproduction [66]. Later, following Weismann's insight, it was highlighted that aging increases evolvability, i.e., the speed of evolution, and so it is favoured by natural selection [60 61]. In possible harmony with the idea that aging is adaptive and programmed, damage by mitochondrial ROS has been proposed as the essential mechanism [58 59 65]. In other papers, although a specific

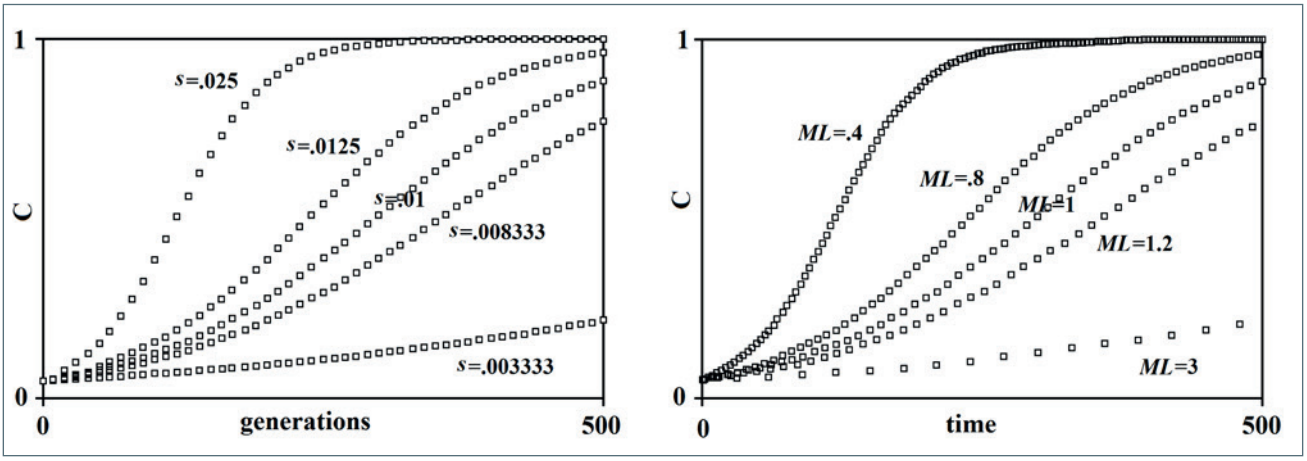

Figure 6. On the left: spreading of C according to the variation of *s* (while *ML* = 1); on the right: spreading of C according to the variation of *ML* (while *s* = 0.01); C_0 = .05. Redrawn from figures 2 and 3 in Libertini, 1988 [1], which are the same of figures I 2-1 and II 2-1 in Libertini, 1983 [51].

theory about aging is not formulated, the idea that this phenomenon is adaptive and programmed is backed with various topics [56 57 62 64 91].

Despite the substantial differences among the various hypotheses about aging interpreted as an adaptive and programmed phenomenon, in 2008, some possible common predictions were highlighted: (i) the existence of non-aging species; (ii) among different species, an inverse relationship between the proportion of senescent deaths and extrinsic mortality; (iii) the existence of genetically determined and regulated mechanisms for aging. Moreover, it was highlighted that: the point (i) was difficult or impossible to explain by many non-programmed aging theories; and the points (ii) and (iii) were incompatible with them [88].

Regarding the various life table types, it is possible to highlight some general distinctions between old and new paradigm hypotheses, which are summarized in Table I and in Figures 7A and 7B.

THE "HOW" OF AGING

For the new paradigm, as aging is considered an adaptive phenomenon, it is predictable and indeed imperative that aging is genetically programmed and regulated by specific mechanisms. On the contrary, for the old paradigm, as aging is a considered a consequence of degenerative processes insufficiently countered by natural selection, the aforesaid mechanisms simply cannot

Table I. Some distinctions between old and new paradigm.

	Species that…	For the old paradigm…	For the new paradigm…
1	Show IMICAW	This is the primary or most primitive condition	This is a particular evolved condition that is favoured only under particular ecological conditions
2	Do not show IMICAW or, prudentially, are defined as "with negligible senescence" (from Finch, 1990 [8])	These are exceptions that must be explained	This is the primary or most primitive condition, not exceptions that must be explained
3	Do not show IMICAW and, in certain periods of the life, even show a decreasing mortality	These are exceptions that must be explained	This is a variant of the primary condition, determined by particular causes (e.g., an increment in body mass that reduces predation)
4	Do not show IMICAW, show very high mortality, very short life spans and IMICAC	These are not exceptions because show IMICAC (which is not distinguished from aging)	These are non-aging species and IMICAC cannot have an evolutionary meaning because cannot be determined by natural selection
5	Do not show IMICAW, but in a certain phase, e.g. in reproduction, show a sudden death	This is a particular type of aging and the absence of IMICAW is disregarded	These are not aging species and their death is a form of phenoptosis, i.e. an adapted condition

Abbreviations: IMICAW: "increasing mortality with increasing chronological age in populations in the wild" (from Libertini, 1988 [1]); IMICAC: "increasing mortality with increasing chronological age in populations in captivity (i.e., under protected conditions at ages non-existing in the wild)" (from Libertini, 1988 [1]).

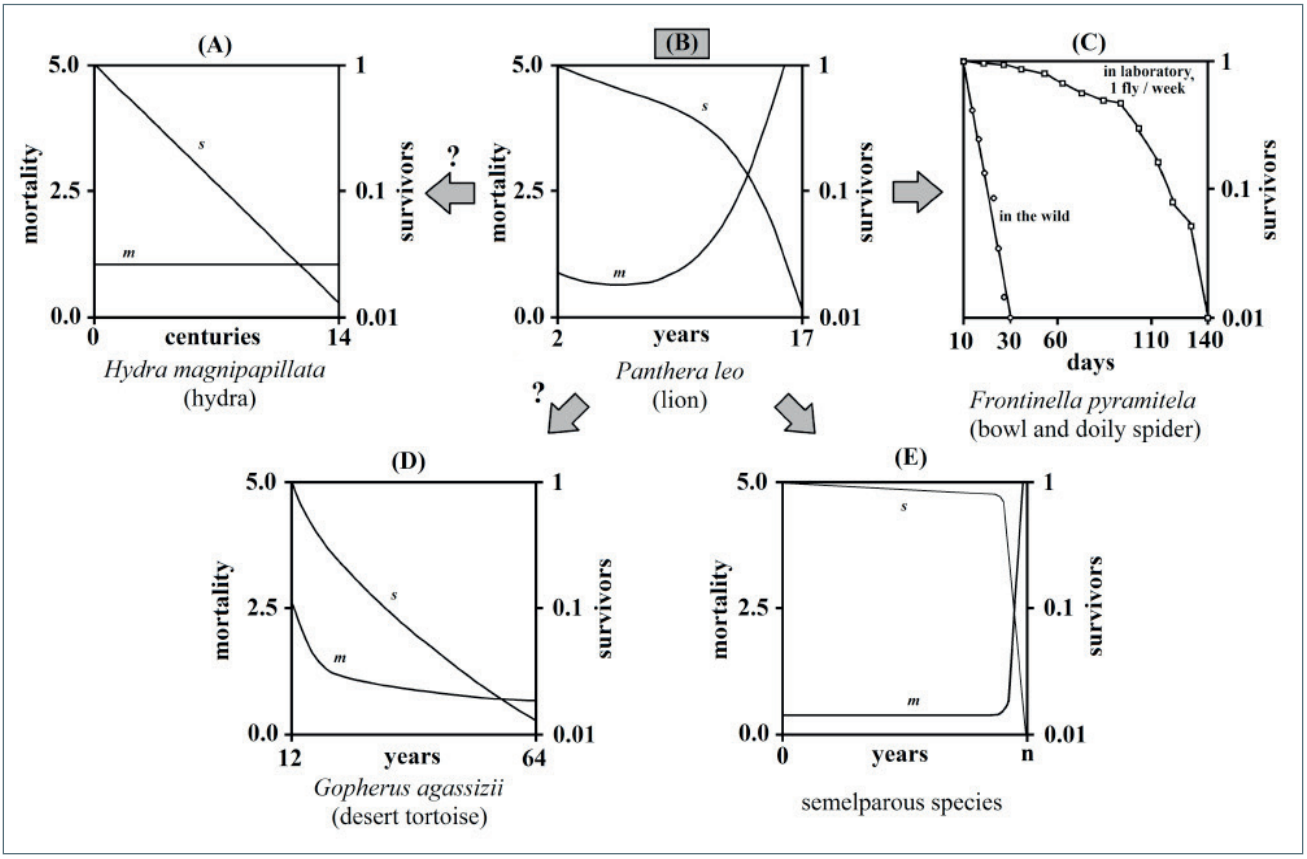

Figure 7A. For the old paradigm, the primary condition is (B) and the other conditions are derived, although (A) and (D) are difficult to explain. (A), (B) and (D) are from Figure 1 of Jones, Scheuerlein, Salguero-Gómez, 2014 [83], partial and redrawn, only mortality (*m*) and survivorship (*s*) are indicated; (C) has been drawn by using data from Austad, 1989 [86]; (E) is an ideal life table of a semelparous species as reported in Finch, 1990 [8].

exist and, so, are indeed in utter contradiction with the paradigm. Also, for the old paradigm, the various degenerative mechanisms proposed as causes of aging represent a description of the "how" of aging.

Beyond the general issues exposed in the previous section, the existence or non-existence of genetically programmed and regulated specific mechanisms that determine aging is a fundamental and definitive evidence to settle the alternative between the old and new paradigm [16]. This section is an overview of aging mechanisms as they are shown by the evidence and highlights that they are necessarily determined and regulated by genes. This description is the result of decades of work by researchers who often were, and are, not supporters or even aware of the new paradigm. On the contrary, these researchers were sometimes influenced, more or less consciously, by the tenets of the old paradigm. As we will see, the new paradigm allows for the interpretation of the experimental results within a consistent and understandable framework, while, for the old paradigm many results appear inexplicable and difficult or impossible to harmonize in a general and consistent theory.

Cell turnover: programmed cell death

In vertebrate species, organisms show a continuous renewal of their cells. Disregarding the cases in which cells die as a result of accidental events, cells usually die through the action of genetically determined and regulated mechanisms that are defined in general as "programmed cell death" (PCD). For example, epidermis cells are transformed by keratinization, die and then become detached; mucosal cells that line the intestine continually come off; erythroblasts transform themselves into erythrocytes and are subsequently removed by macrophages.

Apoptosis is a type of PCD described only in quite recent times that affects healthy tissues previously considered to lack cell turnover [92]. It is ubiquitous in the eukaryotic world [64] and is certainly very old phylogenetically: it is observed, with some differences, even in

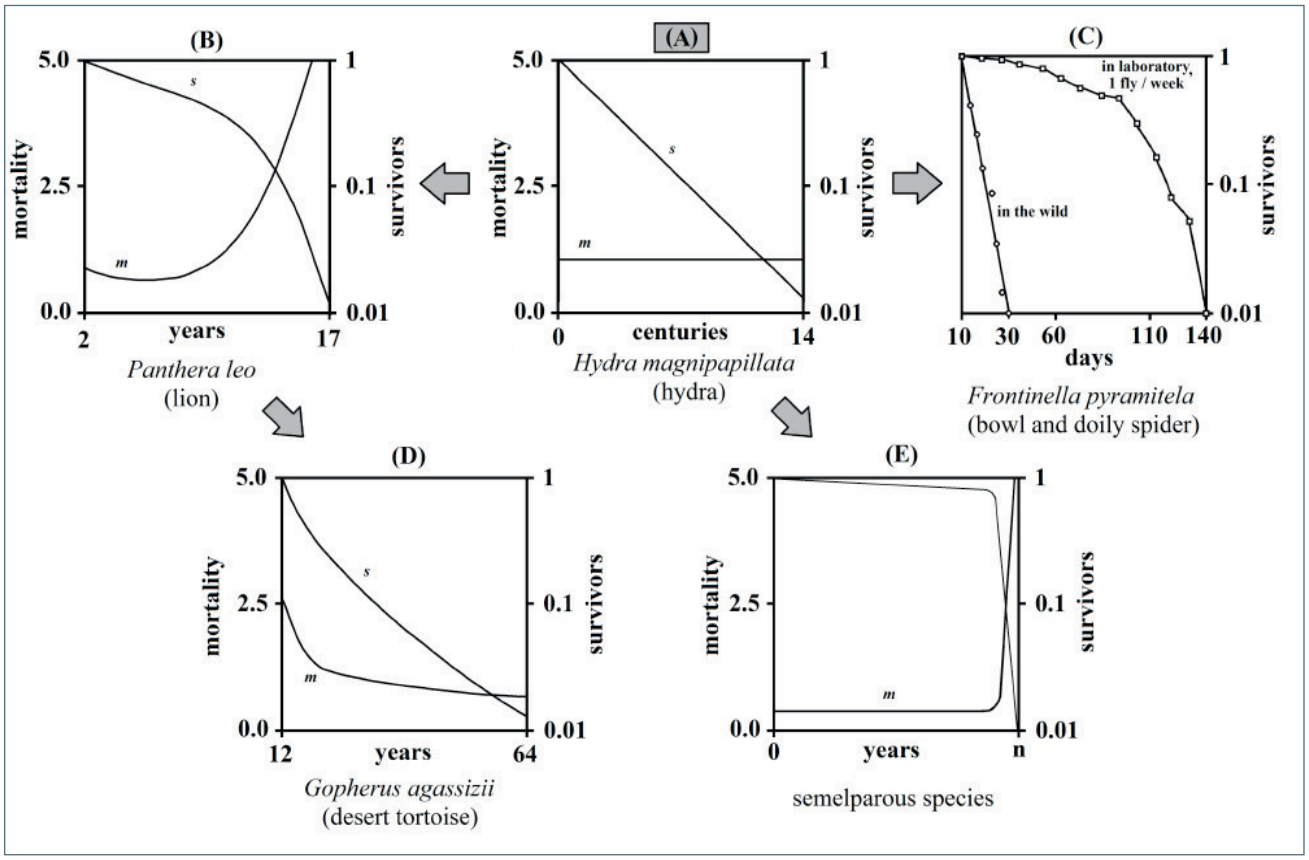

Figure 7B. For the new paradigm, the primary condition is (A) and the other conditions are derived.

unicellular species such as yeast [93]; furthermore, there are similar and phylogenetically related phenomena, defined as "proapoptosis", in prokaryotes [94 95]. Apoptosis is clearly different from necrosis, as it follows an ordered sequence, does not damage other cells and does not trigger an inflammatory response [96]. Apoptosis shows itself in many healthy tissues and organs [97-109] and is essential to ensure cell turnover [110-113], although it has other important functions (e.g.: removal of cells that are injured or infected [114 115], lymphocyte selection [116 117], morphogenetic mechanisms [118], wound healing [119] etc.).

Cell turnover is a massive phenomenon: an estimate for our species is that about 50 to 70 billion cells are eliminated each day by PCD events (580,000 to 810,000 cells per second), i.e., in one year, a mass equal to that of the entire weight of the body [120].

Cell turnover varies greatly in its rhythms depending on organ and cell type [121]. At one extreme we have the cells of colon mucosa that are replaced in 3-6 days [122], at the other extreme "the heart is replaced roughly every 4.5 years" [123] and the "bone has a turnover time of about ten years in humans" [122].

Cell turnover: cell replication and its limits

To compensate for cells eliminated by PCD, cell turnover clearly requires cell replication that, however, is restrained by known mechanisms.

In the late nineteenth century, August Weissmann proposed, without deepening the idea, that the limits to cell replication were an explanation for aging [48 50]. For many years, his insight was considered unsustainable because it was wrongly believed, with the authoritative endorsement of a Nobel prize, that somatic cells of an organism were capable of unlimited replication [124 125]. Many years later, breaking this inveterate prejudice, it was demonstrated, *in vitro*, that the duplication capabilities were limited [126 127]. Later, it was shown that this limitation (Hayflik's limit) was also evident *in vivo* [128] and for many cell types [129-131]. The duplication capacities were shown to be inversely correlated with age [132] and, in the comparison between species, directly correlated with longevity [133]. In 1975, it was shown that something in the nucleus was the cause of the limit [134].

However, it was observed that the linear DNA of eukaryotes was duplicated only partially by the DNA polymerase. During each replication, a small part of one end

of the DNA molecule (telomere) is not replicated [135 136]. As an unlimited shortening was not compatible with the functionality of the cell, it was predicted the existence of an enzyme that had to restore the unduplicated part [137]. In subsequent years, the telomere was shown, in a protozoan, to be a simple repeated sequence of nucleotides (TTGGGG) [138]. The same sequence with minimal variation (TTAGGG) was present in our species and in mammals [139] and in many other species that are phylogenetically distant [140]. In 1985, we identified an enzyme (telomerase) that confirmed Olovnikov's prediction because it added the sequence of non-duplicated nucleotides. This explained the capacity of certain cells, such as stem cells and germ-line cells, to reproduce many or unlimited times [141]. It was later shown that: telomerase is repressed by specific regulatory proteins [142]; telomere length shows, in many cell types, an age-related progressive shortening [143]; in individuals of animal species studied in the wild there is association between life expectancy and telomere length [144-146]; inactivated telomerase and/or short telomeres increase the probability of apoptosis [147-151].

Subtelomere-Telomere-Telomerase system

The telomere is covered by a heterochromatin hood. In cells in which telomerase is inactive, or partially active, as the telomere shortens, the hood slides over the part of the DNA molecule that is adjacent to the telomere (subtelomere) and causes progressive transcriptional silencing of the subtelomere and alters the functions regulated by subtelomere [151]. This repressing effect, which has been known for some time as the "telomere position effect" [152], defined as "gradual senescence" too [75], alters also the functioning of genes placed "over long distances" in the DNA molecule [153] and causes many alterations of cell functions, cellular secretions included (e.g., elastin, collagen etc.), which cause modifications of the intercellular matrix, damages to other cells and inflammation [151].

The hypothesis that the subtelomere has a regulatory function is supported by evidence: (i) the subtelomere has an "unusual structure: patchworks of blocks that are duplicated" [154], (ii) "A common feature associated with subtelomeric regions in different eukaryotes is the presence of long arrays of tandemly repeated satellite sequences" [155]. These repeated sequences are likely to have regulatory functions and are suppressed one after the other by the sliding of the telomere hood.

When the telomere shortens to a critical point, this inevitably triggers a chain of events, called "cell senescence" and defined as a "fundamental cellular program" [156], which involves the inability of the cell to duplicate further (replicative senescence) as well as maximal alterations of gradual senescence.

However, in the culture of cells with equal numbers of previous duplications, there was a progressive reduction of the average capacity of duplication, or growth potential, and not a contemporary collapse in replication capacity of all cells after a certain number of duplications [97 157]. This was later explained by Blackburn [158]: the telomere, which is covered by the aforesaid hood, oscillates between "uncapped" and "capped" conditions. In the first state, there is vulnerability to the transition to replicative senescence, i.e., activation of the cell senescence program. Furthermore, the duration of the "uncapped" state is proportional to the reduction in telomere length, but, even when the telomere is minimally reduced, there is a small uncapped phase and so a small probability that replicative senescence will be triggered.

All this could suggest that the critical element is the "absolute" length of the telomere and that therefore the initial telomere length (i.e., that in the first cell of an organism) is the factor that determines the number of possible duplications and consequently potential longevity. However, the evidence shows: (i) no correlation between telomere length and longevity among different species of rodents [159] and among hamsters, mice and men [160]; (ii) two *Mus* strains with different telomere lengths exhibit the same aging rhythms and equivalent longevity [151], (iii) similarly, for cloned animals derived from somatic cells, i.e., with shortened telomeres, and non-cloned individuals [151]. In fact, the key factor is not the initial "absolute" length of the telomere but rather the progressive inhibition of the subtelomere, which is a function of "relative" telomere shortening and not of its initial "absolute" length [75 151] (Fig. 8).

These phenomena ("gradual senescence" and "cell senescence", which includes "gradual senescence" to its maximum degree) are completely reversed in vitro by the activation of telomerase [161-165]. As "cell senescence" may be completely and quickly triggered or, on the contrary, cancelled, it has also been defined as "on/off senescence" [16 75 166].

Notably, aged fibroblasts in which telomerase was reactivated in vitro were used to form human skin that could not be distinguished from skin reconstituted from young fibroblasts [167].

In vivo, telomerase reactivation: (i) in aged mice with blocked telomerase, showed a clear reversal of all aging manifestations, even those of the nervous system [168], (ii) in one- and two-year-old normal mice, increased lifespan and delayed all aging manifestations [169].

Germ-line cells duplicate without limits and no transformation into senescent cells or manifestation of gradual senescence. On the contrary, these phenomena happen for somatic cells but are completely reversed by telomerase activation. The differences between germ-line

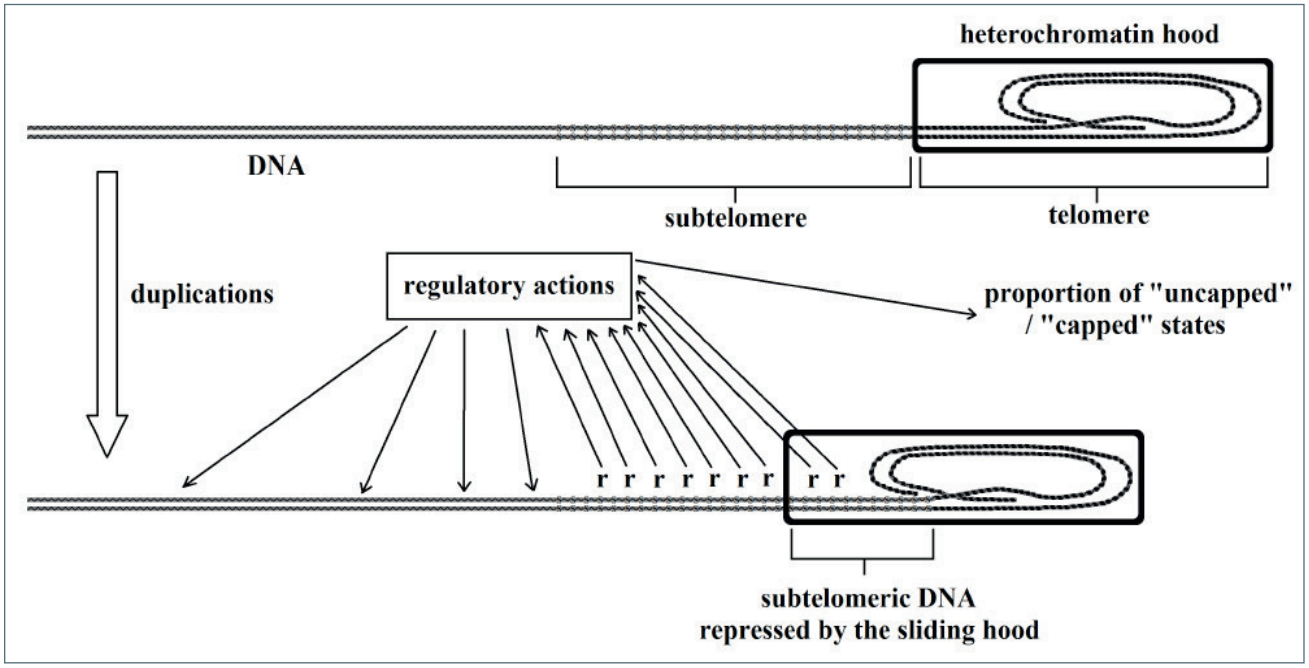

Figure 8. Sliding of the heterochromatin hood over the subtelomere represses an increasing portion of the subtelomere, which probably has repeated regulatory ("r") sequences. This alters gene expression in near and distant parts of the DNA and, moreover, increases the proportion of telomere "uncapped" phase that is vulnerable to the triggering of cell senescence.

and somatic cells and the reversibility of gradual and on/off senescence are hardly explainable by the hypothesis that gradual and on/off senescence are caused by damaging factors, while it is perfectly compatible with the thesis that they are programmed phenomena. This is in clear support of the new paradigm and in clear contrast with the old paradigm.

Effects on the whole organism

The gradual increase in the number of cells that show cell senescence or gradual senescence, the slowing of cell turnover, and the resulting alterations in other cells, cause an "atrophic syndrome" in each organ, tissue and apparatus, already described elsewhere [53]. It is characterized by:

a) reduced number of functional cells;
b) hypertrophy of the remaining functional cells;
c) partial substitution of the lost cells with nonspecific cells;
d) reduced mean cell duplication capacity;
e) slower cell turnover;
f) increasing number of cells in gradual senescence or in cell senescence;
g) increasing cancer risk due to dysfunctional telomere-induced instability [170].

Regarding the cell types without turnover (e.g., most neuron types, crystalline lens fibre cells), they are dependent from cells with turnover and so suffer from the consequences of turnover decline in these cells. This topic has been developed in a recent paper [171] and for brevity will not be repeated.

Through the effects of harmful substances and unhealthy lifestyles, the aging process is accelerated, and, on the contrary, "protective drugs" and healthy lifestyles contrast this acceleration. These topics and a comprehensive description of the aging process for various organs and tissues have been concisely expounded upon elsewhere [87 166]. Figures 9 and 10 are schemes of these concepts.

Aging and cancer

The subtelomere-telomere-telomerase system is the key part of the mechanisms required by the new paradigm to explain aging. At the same time, these mechanisms are utterly incompatible with the old paradigm if there is no alternative evolutionary motivation for their existence. The only (old) explanation proposed is that they are a defence against cancer because replicative senescence would pose an obstacle to neoplastic proliferation [172-174]. So, aging would be an evolutionary necessity to contrast cancer [175], a hypothesis that could be compatible with some theories of the old paradigm (antagonistic pleiotropy theory [25 33], disposable soma theory [29 30]). However, this hypothesis is contrasted by strong arguments [55 87 176], e.g.: (i) telomere shortening increases the probability of cancer [170 177 178], (ii) gradual

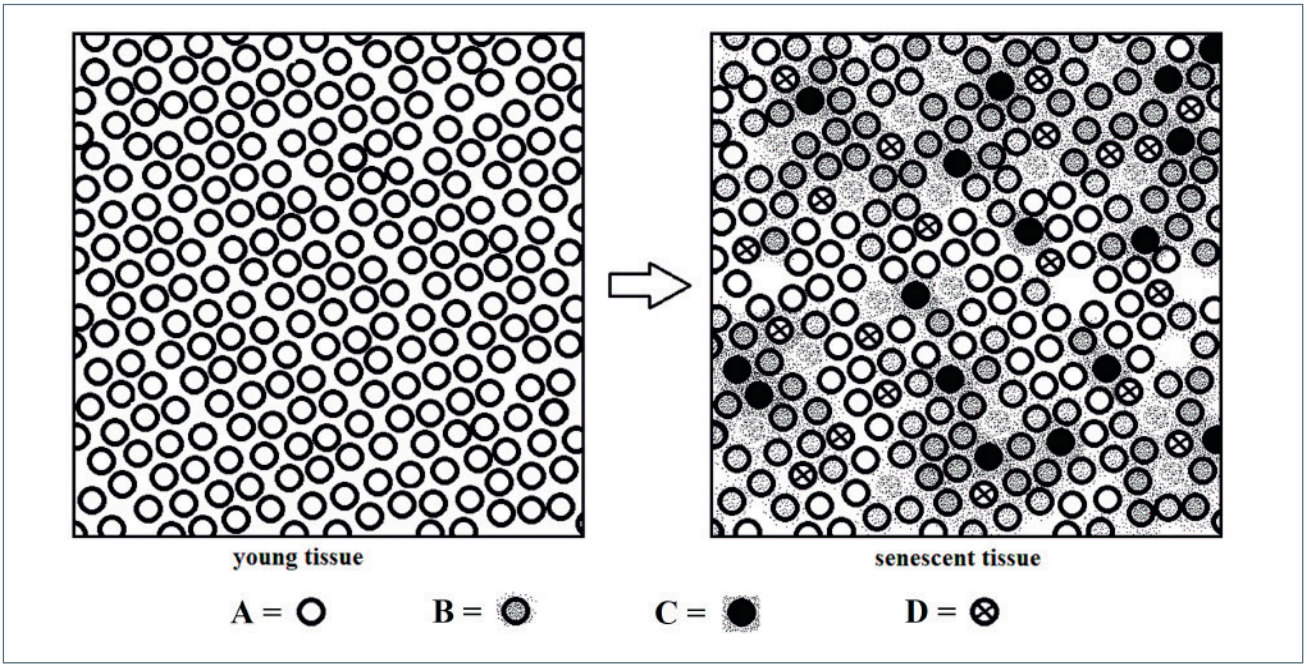

Figure 9. Scheme of the transformation of a young tissue into an old tissue. A: normal cell; B e C = cells in "gradual" and "on/off" senescence with alterations of the surrounding milieu; D = nonspecific substituting cells.

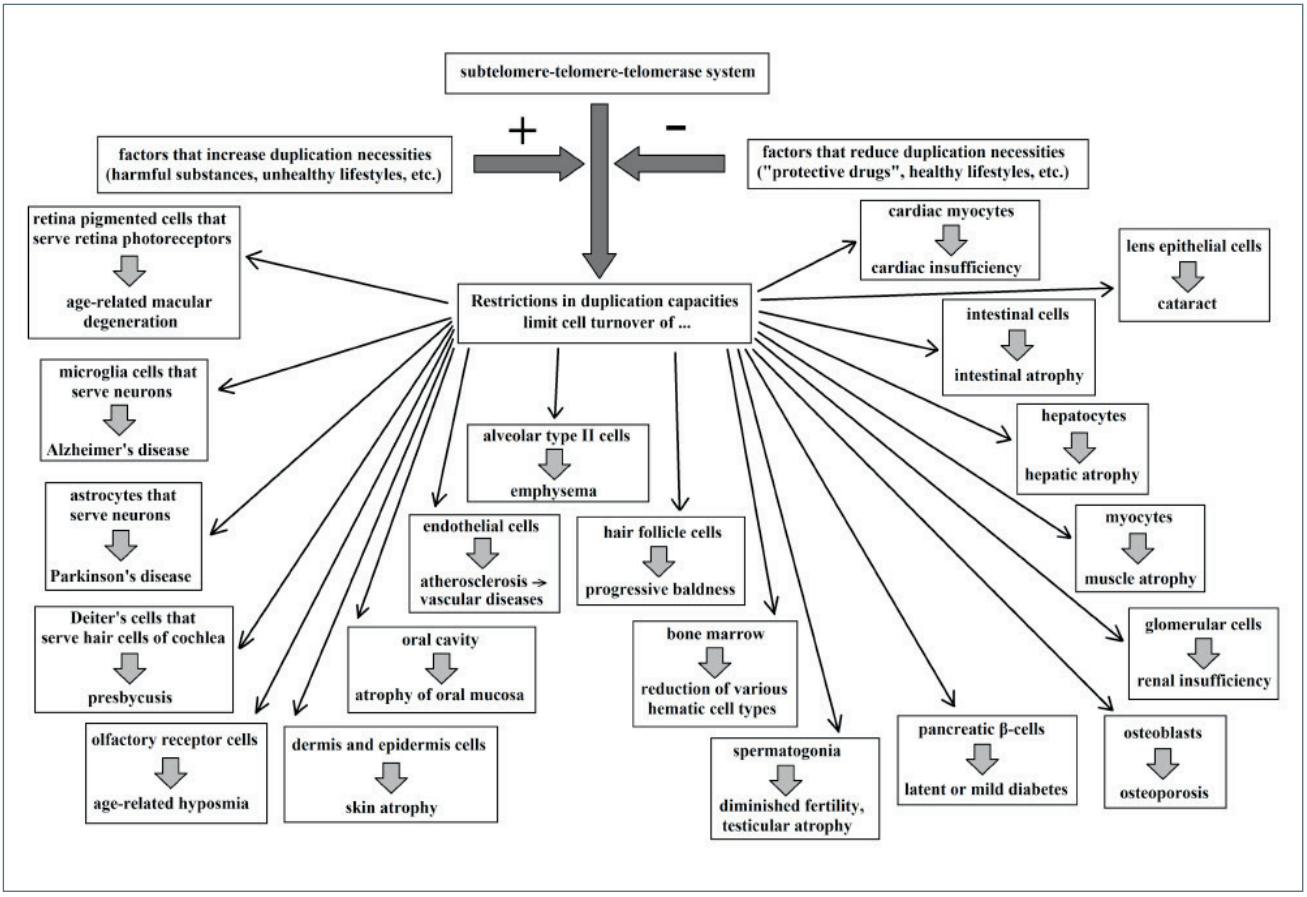

Figure 10. Scheme of aging mechanisms at organismal level.

and on/off senescence weakens immune system efficiency [151] and so increases vulnerability to cancer [179], (iii) old individual "animals with negligible senescence" [8] have the same telomerase activity as young individuals [180 181] without any increased cancer vulnerability as proven by their constant mortality, (iv) in humans, there is relationship between cancer risk and short telomeres [173 182 183], (v) increased expression of telomerase in normal mice increases lifespan and does not cause cancer [169], (vi) "If cellular senescence is designed to cut off cancerous cell lines, why would senescent cells remain alive and toxic?... from the perspective of the cancer theory, the poisoning of the body must be regarded as an unexplained evolutionary error" [176], (vii) in humans studied in the wild, cancer was a possible cause of death only for few older individuals (> 70 years), while most of the deaths were a consequence of the decreasing fitness caused by aging [10]. It is unjustifiable that a hypothetical defence against rare events, which happen at later ages, kills many younger individuals [55].
A recent attempt to explain some of these contradictions within the fence of the old paradigm [174] has been considered insufficient and biased [176].

Pathology of aging

This is a subject concisely discussed in other works [87 166] and, for brevity, cannot be expounded upon here. In general, it is necessary to distinguish between rare diseases originated by genetic alterations (e.g., Werner syndrome [184], dyskeratosis congenita [185]) and frequent or very frequent diseases caused by risk factors resulting from unhealthy lifestyles that accelerate and alter physiological aging. It is important to note the possibility of a distinction between the physiology and pathology of aging in accordance with the predictions of the new paradigm.

Phylogenesis of aging

The phylogenesis of aging has been debated in a recent paper [75] and, for brevity, only a single fact will be highlighted. In yeast (*S. cerevisiae*), telomerase is always active and mother-line cells manifest aging alterations due to increasing subtelomere inhibition caused by the progressive accumulation of particular molecules (ERCs). In daughter-line cells, this does not happen but, in *tlc1Δ* mutants in which telomerase is deficient, the telomere is shortened with each cell duplication and the subtelomere is inhibited by the progressive sliding of the cap on it [186], similarly to what occurs in mammals.

CONCLUSIONS

Among numberless types of phenoptosis, which are all considered adaptive [8 54], it is odd that aging, also defined as "slow phenoptosis" [81 82], is the only one still considered by many as non-adaptive. In 1977, Hayflick wrote: "… if normal animal cells do indeed have only a limited capacity for division in cell culture, then manifestations of aging might very well have an intracellular basis" [187]. As these limits for cell division was later shown to be genetically determined and regulated, this statement could be considered a wise anticipation of the new paradigm.
However, twenty-five years later, an authoritative "position statement", written by the same Hayflick and two other leaders in aging sciences and endorsed by about 50 known worldwide scientists, stated: "No genetic instructions are required to age animals", "… longevity determination is under genetic control only indirectly", "… aging is a product of evolutionary neglect, not evolutionary intent" [188].
The concepts of this "position statement", which is a comprehensive expression of the old paradigm, appear to be strongly contradicted by the arguments and the evidence presented in this review. The same arguments and facts appear to be in accordance with 1977 Hayflick's insight and entirely compatible with the new paradigm.
Therefore, a paradigm shift should be considered necessary and unavoidable.

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Review

Dietary patterns, foods, and food groups: relation to late-life cognitive disorders

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The limited efficacy of disease-modifying therapeutic strategies for mild cognitive impairment (MCI) and Alzheimer's dementia (AD) underscores the need for preventive measures to reduce the burden of late-life cognitive impairment. The aim of the present review article was to investigate the relationship among dietary patterns, foods, and food groups and late-life cognitive disorders considering the results of observational studies published in the last three years (2014-2016). In the last decade, the association between diet and cognitive function or dementia has been largely investigated. However, more recently, the National Institute on Aging-Alzheimer's Association guidelines for AD and cognitive decline due to AD pathology introduced some evidence suggesting a direct relation between diet and changes in the brain structure and activity. Several studies focused on the role of the dietary patterns on late-life cognition, with accumulating evidence that combinations of foods and nutrients into certain patterns may act synergistically to provide stronger health effects than those conferred by their individual dietary components. In particular, higher adherence to a Mediterranean-type diet was associated with decreased cognitive decline, although the Mediterranean diet (MeDi) combines several foods, micronutrients, and macronutrients already separately proposed as potential protective factors against dementia and MCI. Moreover, also other emerging healthy dietary patterns such as the Dietary Approach to Stop Hypertension (DASH) and the Mediterranean-DASH diet Intervention for Neurodegenerative Delay (MIND) diets were associated with slower rates of cognitive decline and significant reduction in AD rate. Furthermore, some foods or food groups traditionally considered harmful such as eggs and red meat have been partially rehabilitated, while there is still a negative correlation of cognitive functions with added sugars and trans fatty acids, nutrients also increasing the cardiovascular risk. This would suggest a genesis for the same damage for aging brain.

Key words: Dementia, Alzheimer's disease, MCI, Dietary pattern, Mediterranean diet, Healthy diet, Foods, Food groups

INTRODUCTION

Currently, available drugs for the treatment of Alzheimer's disease (AD) have only symptomatic effects [1], and there is an unmet need of preventing AD onset and delaying or slowing disease progression from mild cognitive impairment (MCI) in absence of disease-modifying therapies. In the last ten years, a large number of studies have investigated the association between diet and cognitive function and dementia [2-4]. However, in the last few years, some changes have emerged in approaching the relationship between diet and cognitive impairment. In fact, the National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines for AD and cognitive decline due to AD pathology [5] introduced

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some evidence suggesting a direct relation between diet and changes in the brain structure and activity, opening the era of brain imaging biomarkers in nutrition epidemiology. Furthermore, some groups of foods traditionally considered harmful such as eggs and red meat have been partially rehabilitated. Conversely, there is still a negative correlation of cognitive functions with added sugars and trans fatty acids, the same nutrients that increase the cardiovascular risk, suggesting a genesis for the same damage for aging brain. Finally, many studies focused on the role of dietary patterns on late-life cognition, accumulating evidence that combinations of foods and nutrients into certain patterns may act synergistically to provide stronger health effects than those conferred by their individual dietary components. The aim of the present review article was to shed light on the relationship among dietary patterns, foods, and food groups and late-life cognitive disorders considering the results of observational studies published in the last three years (2014-2016).

DIETARY PATTERNS AND LATE-LIFE COGNITION

The Mediterranean diet (MeDi) is a typical dietary pattern of Mediterranean countries, characterized by high consumption of fruits, vegetables, legumes and cereals, olive oil as the main added lipid, moderate consumption of alcohol (mainly wine and during meals) and low consumption of red meat and dairy products. It is doubtless the most analyzed dietary pattern and accumulating evidence support a potential protective role against cognitive decline and dementia, although there are still inconsistencies in the reported data. In particular, the findings from prospective studies and very recent systematic reviews and meta-analyses suggested that adherence to the MeDi fulfilling the whole-diet approach may affect not only the risk of AD, but also of predementia syndromes and their progression to overt dementia.[6] In the last two years, in the EPIC study, in a cohort of Greek elderly population that still adheres to the traditional MeDi, it was demonstrated that closer adherence to MeDi was associated with less decline in Mini Mental State Examination (MMSE) performance over a period of about 7 years, especially in individuals aged 75 years or older (Tab. I) [7].

Other emerging dietary patterns are the Dietary Approach to Stop Hypertension (DASH) and the Mediterranean-DASH diet Intervention for Neurodegenerative Delay (MIND) diets (Tab. I). The DASH diet is characterized by low consumption of saturated fat and commercial pastries and sweets, and higher intake of dairy than in the MeDi. In the last three years, in the Memory and Aging Project (MAP) study, a prospective study on older adults with 4 years of follow-up, the DASH pattern was associated with slower rates of cognitive decline. In particular, a 1-unit-higher DASH score, was equivalent of being at least 4.4 years younger (Tab. I) [8]. These results were in line with those of Morris and colleagues, in the same MAP study, in which higher adherence to DASH diet was related with greater reduction of incident AD rather than higher adherence to MeDi (54% and 39% reduction, respectively) (Tab. I) [9].

The MIND diet was based on the dietary components of the MeDi and DASH diet with modifications that highlight the foods and nutrients shown to be associated with dementia prevention. Among the MIND diet components, there are 10 brain healthy food groups (green leafy vegetables, other vegetables, nuts, berries, beans, whole grains, seafood, poultry, olive oil, and wine) and five unhealthy food groups (red meats, butter and stick margarine, cheese, pastries and sweets, and fried/fast food). Hence, MIND diet uniquely specifies consumption of berries and green leafy vegetables and does not specify high fruit consumption (both DASH and MeDi), high dairy (DASH), high potato consumption, or > 1 fish meal per week (MeDi). Other recent findings from the MAP study suggested that higher MIND diet score was associated with slower decline in cognitive abilities (Tab. I) [10]. The rate reduction for persons in the highest tertile of diet scores compared with the lowest tertile was the equivalent of being 7.5 years younger. MIND diet score was also more predictive of cognitive decline than either of the other (DASH and MeDi) diet scores (Tab. I) [10]. Furthermore, in a follow-up of 4.5 years of the MAP study, participants with higher and moderate adherence to MIND diet had statistically significant reduction in AD rate compared with those with lower adherence (53% and 35% respectively) [9]. Instead only the highest tertiles of the DASH and MeDi scores were significantly associated with incident AD reduction (Tab. I) [9].

Despite the promising results of these two diets, to date, we have brain imaging data only on the correlation with the MeDi (Tab. I). The few cross sectional studies carried out on cognitively normal people showed that higher adherence to MeDi was related to greater magnetic resonance imaging (MRI)-based cortical thickness in AD-vulnerable regions and larger brain volumes. MeDi effects on MRI biomarkers were significant in the left, but not in the right hemisphere, and were most pronounced in entorhinal cortex, orbito-frontal cortex and posterior cingulate cortex (Tab. I) [11]. Higher adherence to a Mediterranean dietary pattern was associated with larger MRI measures of cortical thickness and with several individual region of interests (ROIs) that undergo age-related or AD-related neurodegeneration, was

marginally associated with temporal and AD signature cortical thickness and was not associated with hippocampal volume (Tab. I) [12]. This finding may be explained with the observation from the Alzheimer's Disease Neuroimaging Initiative in which presymptomatic individuals had significantly reduced cortical thickness in AD vulnerable regions compared to controls but did not differ in regard to hippocampal volume [13]. In the Washington Heights-Inwood Community Aging Project (WHICAP), higher MeDi adherence was associated with less brain atrophy (larger total brain volume, total gray matter volume, total white matter volume), with an effect similar to 5 years of aging (Tab. I) [14].

To date, only one prospective imaging-diet study on older adults was conducted (Tab. I) [15], confirming other results coming from cross-sectional studies. In fact, Jacka and colleagues, in the Personality and Total Health Through Life Study found that healthy "prudent" dietary pattern characterized by intake of fresh vegetables, salad, fruit and grilled fish was associated with a larger left hippocampal volume on MRI over 4 years of follow-up (Tab. I) [15]. In particular, every one standard deviation (SD) increase in healthy "prudent" dietary pattern was associated with a 45.7 mm^3 larger left hippocampal volume [15]. While higher consumption of an unhealthy "Western" dietary pattern characterized by intake roast meat, sausages, hamburgers, steak, chips, crisps and soft drinks was independently associated with a 52.6 mm^3 smaller left hippocampal volume [15]. The difference in hippocampal volume between those classified with a healthy and or unhealthy diet was 203 mm^3, a difference which corresponds to 62% of the average decline in left hippocampal volume observed over the 4-year period. It was found no interaction between right hippocampus volumes and the two dietary factor scores (Tab. I) [15].

Other studies suggested a strong impact of healthy diets on structural connectivity in older subjects, rather than gray and white matter volumes. In fact, through diffusion tensor imaging (DTI) at MRI examination was seen that higher adherence to the MeDi was associated with preserved white matter microstructure in multiple brain areas and appeared to delay cognitive aging by up to 10 years (Tab. I) [16]. None of the individual components was strongly associated with DTI parameters, supporting the hypothesis that overall diet quality may be more important to preserve brain structure than any single food. These results suggested the involvement of vascular pathways rather than neurodegenerative mechanisms in the link between the MeDi and lower risks of cognitive decline and related diseases (Tab. I) [16].

The importance of components of prudent dietary pattern (vegetables, fruit, cooking/dressing oil, cereals and legumes, whole grains, rice/pasta, fish, low-fat dairy, poultry and water) was confirmed by the observation that the MMSE decline associated with Western diet may be attenuated by high adherence to prudent pattern (Tab. I) [17]. In fact, the decline became less pronounced (53.5%) and non-significant among people who had a high adherence to both the prudent and Western patterns. Furthermore, Western dietary pattern score was significantly associated with all-cause mortality in the older age cohorts (Tab. I) [17]. Instead, people who followed healthiest diet were slightly older, more active, less likely to smoke, had a lower body mass index (BMI), normal serum creatinine, and had higher MMSE score (Tab. I) [18]. The healthiest diet was associated with a reduction of about 24% in risk of cognitive decline and in particular was shown a significant association between higher diet quality and reduced risk of decline in 4 components of the MMSE including copying, attention and calculation, registration and writing (Tab. I) [18]. The brain damage related to an unhealthy diet may be based on a pro-inflammatory mechanism. Ozawa and colleagues detected an inflammatory dietary pattern (IDP) characterized by higher intake of red meat, processed meat, peas and legumes and fried food, and lower intake of whole grains which correlated with elevated interleukin(IL)-6 (Tab. I) [19]. It was related with greater decline in reasoning and in global cognition and, in a cross-sectional analysis at baseline, a two times greater risk of having a decline of 3 points or more in MMSE (Tab. I) [19].

FOODS, FOOD GROUPS, AND LATE-LIFE COGNITION

Fish and seafood

The emerging data from the last studies on the correlation between fish and seafood consumption and cognitive decline are conflicting. Significant correlations were found in some particular population subgroups [≥ 65 years and apolipoprotein E (*APOE*) ε*4* carriers]. Age significantly modified the association between fish consumption and cognitive change (Tab. II) [20]. In fact, no association was observed among adults aged 55-64 years. Conversely, adults aged ≥ 65 years, that consuming ≥ 1 servings/week fish (i.e., 100 g) had a reduction of cognitive decline rate [20]. Compared with individuals who consumed < 1 serving/week fish, the mean annual rate of global cognitive decline was reduced by 0.35 point equivalent to the disparity associated with 1.6 years of age. Removing shellfish and/or preserved fish from the total fish did not appreciably alter the results (Tab. II) [20].

Interestingly, Morris and colleagues showed that, in *APOE* ε*4* carriers, seafood consumption ≥ 1 meals/

Table I. Observational studies on the relationship among dietary patterns and late-life cognitive disorders (2014-2016).

References	Study design	Sample	Outcome	Cognitive and nutritional assessment	Principal results
Dietary patterns					
Trichopoulou et al., 2015 [7]	Prospective cohort study Follow-up: average 6.6 years	n = 401 older subjects from EPIC-Greece cohort (mean age 74 years)	Association of MeDi or any particular MeDi component with cognitive decline	FFQ (150 items) MeDi score MMSE; MMSE change (cMMSE)	Decline in MMSE performance inversely associated with adherence to traditional MeDi. Only vegetable consumption, showed significant inverse association with cognitive decline
Tangney et al., 2014 [8]	Prospective cohort study Follow-up: mean of 4.1 years	n = 826 older persons (mean age 81.5 years)	Association between DASH diet or MeDi and cognitive decline	MAP FFQ (144 item) DASH diet MeDi Score Global composite score of 19 cognitive tests	DASH and MeDi patterns associated with a slower rate of global cognitive decline
Morris et al., 2015 [9]	Prospective cohort study Follow-up: mean of 4.5 years	n = 923 participant, ages 58 to 98 years	Association of MIND diet, DASH diet and MeDi with incident AD	AD diagnosis at each annual evaluation FFQ (144 items) MIND diet score DASH diet score MeDi score	High adherence to all three diets may reduce AD risk. Moderate adherence to the MIND diet may also decrease AD risk
Morris et al., 2015 [10]	Prospective cohort study Follow-up: mean of 4.7 years	n = 960 participants (mean age 81.4 years)	Association of MIND diet score with cognitive decline Comparing the estimated effects of MIND diet to those of the MeDi and DASH diet	Annual cognitive assessments, global and composite scores of 5 domains FFQ (144 items) at each annual clinical evaluation MIND diet score DASH diet score MedDiet score	The MIND score positively associated with slower decline in global cognitive score and with each of five cognitive domains. The MIND diet score more predictive of cognitive decline than either of the other diet scores
Mosconi et al., 2014 [11]	Cross-sectional study	n = 52 clinically and cognitively normal subjects (mean age 54 years)	Associations between adherence to a MeDi and structural MRI-based brain atrophy in key regions for AD	Semiquantitative FFQ (61-item) MeDi score MRI CT measures for 5 ROIs	Subjects with higher MeDi adherence showed greater thickness of AD-vulnerable ROIs as compared to subjects with lower MeDi adherence
Staubo et al., 2016 [12]	Cross-sectional study	n = 672 cognitively normal participants (mean age: 79.8 years)	Association of MeDi score and MeDi components with MRI measures of CT for the four lobes separately and averaged	FFQ (128 items) MeDi score MRI CT measures	Higher MeDi score associated with larger CT. Higher legume, fish, vegetables, whole grains or cereals intakes were associated with larger CT
Gu et al., 2015 [14]	Cross-sectional study	n = 674 elderly adults without dementia (mean age 80.1 years)	Association between higher adherence to MeDi with larger MRI measured brain volume or CT	FFQ MeDi score MRI scans for TBV, TGMV, TWMV and mCT	Higher MeDi adherence associated with larger TBV, TGMV and TWMV. Higher fish intake associated with larger TGMV and mCT. Lower meat intake associated with larger TGMV and TBV

▶

References	Study design	Sample	Outcome	Cognitive and nutritional assessment	Principal results
Dietary patterns					
Jacka et al., 2015 [15]	Prospective cohort study Follow-up: 4 years	n = 255 older adults (mean age 62.6 years)	Association between dietary patterns and hippocampal volume Association between diet and differential rates of hippocampal atrophy over time	FFQ "Prudent" (healthy) diet and "Western" (unhealthy) diet. Two MRI scans	Lower intakes of nutrient-dense foods and higher intakes of unhealthy foods each independently associated with smaller left hippocampal volume. No evidence that dietary patterns influenced hippocampal volume decline
Pelletier et al., 2015 [16]	Prospective cohort study Follow-up: MRI performed mean of 8.9 years after dietary assessment	n = 146 non-demented participants (mean age 73.0 years)	Association between higher adherence to the MeDi and preserved brain GM volume and WM microstructure	FFQ (148 items) MeDi score MRI Brain GM and WM volumes, and WM microstructure Cognitive assessment	Adherence to the MeDi significantly associated with preserved WM microstructure in extensive areas, a gain in structural connectivity related to strong cognitive benefits
Shakersain et al., 2016 [17]	Population-based longitudinal study Follow-up: 6 years.	n = 2223 dementia-free older adults (mean age 70.6 years)	Impact of dietary patterns on cognitive decline	MMSE Semiquantitative FFQ (98 items) Two dietary patterns: 1) the "Western", 2) the "prudent"; factor scores for each dietary pattern categorized into quintiles	Highest adherence to prudent pattern related to less MMSE decline, whereas the highest adherence to Western pattern was associated with more MMSE decline. Decline associated with Western diet, attenuated by high adherence to prudent pattern
Smyth et al., 2015 [18]	Prospective cohort study Follow-up: 56 months	n = 27860 patients (mean age 66.2 years)	Association of dietary factors and cognitive decline in a population at high risk of cardiovascular disease	MMSE FFQ (20 items) mAHEI	Highest quintile of mAHEI (healthiest diet) associated with a reduced risk of cognitive decline
Ozawa et al., 2016 [19]	Prospective cohort study Follow-up: 10 years	n= 5083 patients (mean age 56 years)	Investigate dietary patterns associated with inflammation Association of such diet with cognitive decline	Alice Heim 4-I, short-term verbal memory, phonemic and semantic fluency, MMSE FFQ (127 item) Serum IL-6 IDP	Dietary pattern with higher intake of red and processed meat, peas, legumes and fried food, and lower intake of whole grains associated with higher inflammatory markers and accelerated cognitive decline

Abbreviation: MeDi: mediterranean diet; FFQ: food frequency questionnaire; MMSE: mini-mental state examination; DASH: dietary approach to stop hypertension; MIND: mediterranean-DASH diet intervention for neurodegenerative delay; AD: Alzheimer's disease; MRI: magnetic resonance imaging; CT: cortical thickness; ROI: region of interest; TBV: total brain volume; TGMV: total gray matter volume; TWMV: total white matter volume; mCT: mean cortical thickness; GM: gray matter; WM: white matter; mAHEI: modified alternative healthy eating index; IL-6: interleukin-6; IDP: inflammatory dietary pattern

week was correlated with lesser burden of brain AD neuropathology, including lower density of neuritic plaques, less severe and widespread neurofibrillary tangles, and lower neuropathologically defined AD (Tab. II) [21]. Furthermore, some studies demonstrated an association between fish consumption and MRI biomarkers (Tab. I) [12 14]. In the Mayo Clinic Study of Aging, higher fish intake was associated with larger cortical thickness summary measures for parietal and average lobar cortical thickness and marginally associated with AD signature cortical thickness, temporal and frontal cortical thickness, and also associated with several individual

Table II. Observational studies on the relationship among foods, food-groups, and late-life cognitive disorders (2014-2016).

References	Study design	Sample	Outcome	Cognitive and nutritional assessment	Principal results
Foods and food-groups					
Qin et al., 2014 [20]	Prospective cohort study Follow-up: mean 5.3 years.	n = 1566 community-dwelling adults (mean age 63 years).	Association of fish consumption with decline in cognitive function.	Diet measured by 3-d 24-h recalls TICSm: global and composite cognitive scores	Age significantly modified the association between fish consumption and cognitive change. At least 1 serving/wk fish predicted slower cognitive decline among ≥ 65 years
Morris et al., 2016 [21]	Cross-sectional analyses	n = 286 autopsied brains (mean age at death 89.9 years)	Relation of seafood consumption with brain mercury levels Association of seafood consumption or brain mercury levels with brain neuropathologies	Brain autoptical assessment Mercury and selenium brain tissue concentrations FFQ for consumption of seafood and n-3 fatty acids in the 4.5 years before death	Seafood consumption (> 1 meal[s]/week) significantly correlated with less AD pathology. Seafood consumption correlated with higher brain levels of mercury, these levels not correlated with brain neuropathology
Danthiir et al., 2014 [22]	Cross-sectional study	n = 390 community-dwelling cognitively normal older adults (mean age 73.1 years)	Associations between multiple domains of cognition and erythrocyte membrane n-3 PUFA proportions and historical and contemporary fish intake in older adults	n-3 FA analysis in erythrocyte membranes Fish consumption (current: FFQ, historical: LDQ) Cognitive tests	No evidence that higher proportions of long-chain n-3 fatty acids or fish intake benefits cognitive perfomances. Negative effect of fish intake in childhood and older age on older-age cognitive functions
Dong et al., 2016 [23]	Cross-sectional study	n = 894 Chinese adults, normal and with mild cognitive impairment (mean age 62.9 years)	Association between nuts, vegetables and fruit-rich diet and the risk of cognition impairment	MoCA FFQ of 13 food groups totally 41 items	The nuts and cooking oil intake of MCI patients were less than the normal subjects. Fruit and vegetable intake will benefit orientation, name and attention ability. Fruit and vegetable juice drinking will benefit abstraction ability
Pastor-Valero et al., 2014 [24]	Cross-sectional population-based study	n = 1849 low-income elderly subjects with CI (n = 147, mean age 77.5 years) and without (n = 1702, mean age 71.5 years)	Association between fruit and vegetable intake and cognitive impairment	CSI-D FFQ: 10 vegetables items, and 17 fruit and natural juices items Monthly consumption of fish	Daily intakes of fruit and vegetable ≥ 400 grams/day associated with decreased prevalence of cognitive impairment. Fish consumption not associated with cognitive impairment
Zhao et al., 2015 [25]	Cross-sectional study	n = 404 patients, aged 60 years old or above, with or without MCI	Association of dietary and lifestyle patterns with MCI	MoCA FFQ	Higher daily intake of eggs and marine products significantly decreased odds of suffering from MCI

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References	Study design	Sample	Outcome	Cognitive and nutritional assessment	Principal results
Foods and food-groups					
Xu et al., 2015 [26]	Cross-sectional study	n = 517 Chinese elderly with possible dementia (22.1%, mean age 73.8 years) and without CI (77.9% mean age 65.7 years)	Effect of weekly tofu intake on cognitive performance	HVLT IR FFQ	High intake of tofu negatively related to cognitive performance. Consumption of meat and green vegetables independently associated with better memory function
O'Brien et al., 2014 [28]	Population-based prospective cohort study. Follow-up: 6 years	n = 16010 women without a history of stroke (mean age 74 years); final sample n = 15467	Association of long-term intake of nuts with cognition	FFQ TICS, immediate and delayed recalls, category fluency, delayed recall of the TICS 10-word list and the digit span backwards test	Increasingly higher total nut intake (≥ 5 nuts/week vs never < 1/month) related to increasingly better overall cognition at older ages
Solfrizzi et al., 2015 [30]	Population-based prospective cohort study Follow-up: 3.5 years	5632 subjects, aged 65-84 year old; final sample n = 1445	Association between change or constant habits in coffee consumption and the incidence of MCI	FFQ MCI diagnosis	Cognitively normal older individuals who increased their coffee consumption had a higher rate of developing MCI, while a constant in time moderate coffee consumption was associated to a reduced rate of the incidence of MCI
Araújo et al., 2015 [31]	Cross-sectional study	n = 14563 public service workers (mean age 51.9 years)	Relation of coffee consumption to performance on specific domains of cognition	Cognitive tests from CERAD battery FFQ Type of coffee, caffeine content, additional items added	Coffee consumption associated with better cognitive performance on memory and efficiency of searching in long-term memory only in elderly, but without a dose response relationship
Beydoun et al., 2014 [32]	Prospective cohort study Follow-up: ~2 visits/person each ~2 years intervals	n= 628-1305 subjects free of dementia (mean age 66.8 years)	Association of caffeine and alcohol intake with cognitive performance	MMSE, BVRT, CVLT, VFT-L, VFT-C, TMT A and B, DS-F, DS-B 7-d dietary records for caffeine and alcohol intakes NAS	Stratum-specific associations by sex and baseline age, between caffeine and alcohol intake and cognition. Putative beneficial effects of caffeine and NAS on global cognition, verbal memory, and attention, and mixed effects of alcohol on letter fluency, attention, and working memory

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References	Study design	Sample	Outcome	Cognitive and nutritional assessment	Principal results
Foods and food-groups					
Travassos et al., 2015 [33]	Cross-sectional multicentre study	n = 88 patients with AD (58%) or MCI (42%) (mean age 66.3 years)	Association of caffeine consumption with the CSF biomarkers, particularly Aβ	FFQ Caffeine and main active metabolites in the CSF and plasma $Aβ_{1-42}$, total tau and phosphorylated tau in the CSF	Caffeine consumption not modify the levels of CSF biomarkers. Theobromine associated with a favorable Aβ profile in the CSF
Kesse-Guyot et al., 2014 [34]	Prospective cohort study Follow-up: mean 13.6 years	n = 2983 middle-aged adults from the SU.VI.MAX 2 study (mean age at cognitive evaluation 65.5 years)	Association between a CDP and cognitive performance	Plasma concentrations of carotenoids 24 h dietary record every 2 months RRR statistical method 6 neuro-psycholo gical tests	Positive correlation between CDP and consumption of orange- and green-coloured fruits and vegetables, vegetable oils and soup. Positive association between a CDP and cognitive function (executive functioning and episodic memory)

Abbreviation: TICSm: telephone interview of cognitive status modified; FFQ: food frequency questionnaire; AD: Alzheimer's disease; PUFA: polyunsaturated fatty acids; LDQ: lifetime diet questionnaire; MoCA: Montreal cognitive assessment; MCI: mild cognitive impairment; CSI-D: community screening instrument for dementia; HVLT IR: Hopkins verbal learning test immediate recall; CERAD: consortium to establish a registry for Alzheimer's disease; MMSE: mini-mental state examination; BVRT: Benton visual retention test; CVLT: California verbal learning test; VFT-L and VFT-C: verbal fluency tests - letter and categorical; TMT A and B: trail making test parts A and B; DS-B and DS-F: digit span forward and backward tests; NAS: nutrient adequacy score; CSF: cerebrospinal fluid; Aβ: β-amyloid; CDP: carotenoid-rich dietary pattern; RRR: reduced rank regression

cortical thickness measures: precuneus, superior parietal, posterior cingulate, supramarginal, middle temporal, and inferior parietal and marginally associated with fusiform CT [12]. Higher fish consumption was also related with larger total gray matter volume [14].

Fish consumption was associated with a slower decline in composite and verbal memory scores (Tab. II) [20]. Other studies did not suggest evidence that higher fish intake may impact positively cognitive performance in cognitively normal older adults [24 25] or in those with cognitive impairment (Tab. II) [23 24]. However, Dong and colleagues found that cognitively normal Chinese older subjects consumed more fish than mild cognitive impairment (MCI) subjects [23] and Zhao and colleagues found that higher consumption of marine products was associated with a significantly decreased probability of suffering from MCI (Tab. II) [25]. Of note, Danthiir and colleagues demonstrated that more frequent consumption of total fish (oily and white) was associated with slower cognitive speed for the constructs of inhibition, simple/choice reaction time, reasoning speed, and memory scanning (Tab. II) [22]. More frequent consumption of oily fish significantly associated with worse inhibitory processes, similarly, consumption of white fish significantly and negatively predicted simple/choice reaction time (Tab. II) [22]. Danthiir and colleagues [22] hypothesized that the negative trends observed between cognitive performance and fish consumption were due to neurotoxic contaminants in fish, such as methylmercury. However, as seen above, Morris and colleagues found that higher brain levels of mercury were not correlated with brain neuropathology (Tab. II) [21].

Fruit and vegetables

In Greece, in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, among the components of MeDi, only vegetable consumption exhibited a significant inverse association with cognitive decline (Tab. I) [7]. The diet-low in fruit and vegetable might increase the risk of cognitive function decline in older adults (Tab. II) [23]. In fact, adherence to WHO recommendations for daily intakes of fruit and vegetable, that are eating 5 or more portions of fruit and/or vegetables a day (≥ 400 g/day), were significantly associated with a 47% decreased prevalence of cognitive impairment (Tab. II) [24]. In contrast to these findings, Xu and colleagues found that among older adults (≥ 68 years of age) being vegetarian (not eating meat), the risk for cognitive impairment increased almost 4-fold (Tab. II) [26]. Imaging data in older cohort showed that higher intake of total vegetables was associated with larger dorsolateral prefrontal and superior parietal cortical thickness,

while vegetables without legumes were associated with larger middle temporal, superior parietal, and dorsolateral prefrontal cortical thickness (Tab. I) [12]. In contrast, fruit consumption was negatively associated with inferior parietal, supramarginal, superior parietal, parietal, and precuneus cortical thickness (Tab. I) [12]. These findings are in keeping with result of another study in which higher fruit intake was associated with lower temporal and hippocampal volumes (Tab. I) [14]. This is probably due to high content of simple sugars and a high glycemic index of several fruits and so the effects of carbohydrate component on increased risk of MCI [27]. In older adults, fruit intake would benefit name ability and attention level, while vegetables intake would benefit orientation ability (Tab. II) [23]. Finally, consumption of green vegetables was independently associated with better memory function and among older elderly (≥ 68 years of age) it reduced the risk for cognitive impairment by almost 20% (Tab. II) [26].

Nuts

Nuts are rich in polyunsaturated fatty acids (PUFA) (omega 3 and 6) and monounsaturated fatty acids (MUFA), and also contain a significant amount of minerals such as phosphorus, potassium, magnesium, calcium, iron and sulfur, and vitamin such as B1, B2, B6 and E. It was found the nut intake of MCI patients was less than that of cognitively normal subjects (Tab. II) [23]. In fact, a study performed on older women found that higher total nut intake (i.e., ≥ 5/week) over the long term was associated with modestly better cognitive performance (Tab. II) [28]. Increasingly higher total nut intake was related to increasingly better overall cognition at older ages. Considering that one year of age was associated with a mean decline of 0.04 standard units on both the global and verbal composite scores, therefore, the mean differences comparing the highest to lowest categories of nut intake were equivalent to approximately two years of cognitive aging (Tab. II) [28]. In the same study, it was found a suggestion that those who consumed walnuts 1 to 3 times per month had better cognition than those who consumed walnuts less than once per month, but there was no overall trend of increasingly better cognitive performance with increasing walnut intake (Tab. II) [28]. Dong and colleagues also showed that nut intake would benefit delayed memory (Tab. II) [23].

Coffee and caffeine intake

As summarized in a recent systematic review, several cross-sectional and longitudinal population-based studies suggested a protective effect of coffee, tea, and caffeine use against late-life cognitive impairment/decline, although the association was not found in all cognitive domains investigated and there was a lack of a distinct dose-response association, with a stronger effect among women than men [29]. The findings on the association of coffee, tea, and caffeine consumption or plasma caffeine levels with incident MCI and its progression to dementia were too limited to draw any conclusion [29]. Furthermore, for dementia and AD prevention, some studies with baseline examination in midlife pointed to a lack of association, although other case-control and longitudinal population-based studies with briefer follow-up periods supported favorable effects of coffee, tea, and caffeine consumption against AD [29]. Recent findings from the Italian Longitudinal Study on Aging (ILSA) suggested that cognitively normal older individuals who increased their coffee consumption had a higher rate of developing MCI, while a constant in time moderate coffee consumption was associated to a reduced rate of the incidence of MCI (Tab. II) [30]. Among older adults in Brasil, coffee consumption was associated with better cognitive performance on memory and efficiency of searching in long-term memory (drinking 2-3 cups of coffee per day was associated with about a 3% increase in the mean number of words remembered on the learning, recall and word recognition tests) (Tab. II) [31]. Also, drinking ≥ 3 cups/day of coffee was associated with an increase of about 1.23 words in the mean number of words pronounced in the semantic verbal fluency test [31]. However, in this Brasilian study, Araujio and colleagues did not find indication of a dose response relationship in these associations [31]. In another Chinese study on cognitively normal and MCI adults, no significant association was detected between drinking of coffee and cognitive function (Tab. II) [23]. Another aspect of coffee assumption is the role of its component such as the caffeine. Coffee is a rich source of caffeine, which acts as a psychoactive stimulant. In a cross sectional analysis, Beydoun and colleagues found that caffeine intake was associated with better global cognitive function (MMSE) at baseline for patients ≥ 70 years (Tab. II) [32]. However, in a study that evaluated the association of caffeine consumption with the cerebrospinal fluid (CSF) biomarkers, particularly β-amyloid (Aβ), in AD and MCI patients, no significant difference was found in daily consumption of caffeine between MCI and AD patients, with no correlation between caffeine consumption and Aβ42 in the CSF (Tab. II) [33]. In the same study, theobromine, xanthine formed upon caffeine metabolism and also directly ingested from chocolate products, was associated with a favorable Aβ profile in the CSF (Tab. II) [33]. Interestingly, theobromine in the CSF did not correlate with caffeine consumption, theobromine consumption, or the levels of caffeine and other xanthines in the plasma, but instead it correlated with levels of caffeine, theophylline, and paraxanthine in the CSF, suggesting that it may be formed by central metabolic pathways [33].

Eggs

Eggs have a high content of proteins and lipids in particular cholesterol. For this reason, they are traditionally considered an unhealthy food. However, eggs have also a significant amount of vitamins A, B6, B12, riboflavin, folic acid, choline, iron, calcium, phosphorus and potassium.
In a recent study, higher daily intake of eggs reduced of about 3% the odds of suffering from MCI (Tab. II) [25]. Instead, in the Chinese study of Dong and colleagues, no significant association was detected between intake of eggs with cognitive function in normal and MCI adults (Tab. II) [23].

Tofu

Tofu is a common food in most of the Far East. It is obtained from curdling of the juice extracted from soybeans. It has a high proteins and PUFA content. Higher weekly intake of tofu was associated with worse memory performance, furthermore among older elderly (≥ 68 years of age), high tofu intake increases the risk (of almost 30%) of cognitive impairment indicative of dementia (Tab. II) [26].

Meat

Red meat is a classical element of Western diet that, as mentioned previously, was associated with worse cognitive performance in several studies (Tab. I) [15 17 19]. Consistent with these findings, a negative association of red meat with inferior and superior parietal cortical thickness was found (Tab. I) [12]. However, this concept should be partially reviewed. In fact, in the last years, eating meat (not being vegetarian) was independently associated with better memory function and in older age (≥ 68 years of age) with a four-fold decrease in risk of possible dementia (Tab. II) [26]. Furthermore, Staubo and colleagues also observed that higher red meat intake was associated with larger entorhinal cortical thickness (Tab. I) [12]. This it could relate to some beneficial components of lean red meat (iron, protein, MUFA, PUFA, cobalamine) and beneficial effects in increasing satiety and reducing weight gain. In the Chinese study of Dong and colleagues, no significant association was detected between intake of light or red meat with cognitive function in normal and MCI adults (Tab. II) [23].

Oil

Dong and colleagues, in their Chinese cohort, found that oil intake of MCI patients was less than the normal subjects (29.76 *vs* 35.20 mL cooking oil per day), in particular would have a positive impact on visual-spatial ability (Tab. II) [23]. Vegetable oils are rich in carotenoids, and in the Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) study carotenoids were associated with higher cognitive performance (Tab. II) [34]. Extra-virgin olive oil (EVOO) is one of the main elements of MeDi, and clinical trials and population studies indicated that this dietary pattern and its main lipid component EVOO could have a protective role against AD [35].

Legumes

Dong and colleagues, in their Chinese cohort, showed that normal subjects consumed more legumes and legume products than MCI subjects, demonstrating that intake of legumes and legume product would benefit overall cognition level (Tab. II) [23]. These data were confirmed by imaging biomarkers, in fact, Staubo and colleagues also found that higher intake of legumes was associated with larger parietal and occipital cortical thickness, and with larger thickness in ROIs for superior parietal, inferior parietal, precuneus, and lingual cerebral cortex (Tab. I) [12].

Grain

In their imaging biomarker study, Staubo and colleagues also showed that intake of whole grains or cereals was associated with larger temporal pole and superior temporal cortical thickness (Tab. II) [12]. Conversely, lower intake of whole grains was associated with higher inflammatory markers (IL-6) and accelerated cognitive decline at older age in the Whitehall II prospective cohort study (Tab. II) [19]. However, in the Chinese cohort of Dong and colleagues, no significant association was detected between intake of whole grain and cognitive function in normal and MCI adults (Tab. II) [23].

Alcohol

Recent findings from the Baltimore Longitudinal Study of Aging suggested that alcohol intake was associated with slower improvement on letter fluency and global cognition among those aged < 70 years at baseline (Tab. II) [32]. Conversely, alcohol intake was associated with better attention and working memory performance, particularly among men and individuals ≥ 70 years at baseline (Tab. II) [32]. Compared with moderate consumption (14 to 28 g/d), individuals with higher alcohol intake (> 28 g/d) had faster decline or slower improvement on the MMSE, particularly among women and in the older group. Overall, among men, and for those aged ≥ 70 years, lower alcohol intake (< 14 g/d) compared with moderate consumption (14 to 28 g/d) was associated with poorer performance in working memory (Tab. II) [32]. In the younger group, consuming < 14 g/day was associated with slower decline or faster improvement in the letter fluency compared with a moderate intake of 14 to 28 g/day. Similar pattern was showed also for attention and executive functioning (Tab. II) [32].

CONCLUSIONS AND FUTURE DIRECTIONS

In the last three years, the association between diet and cognitive function or dementia has been largely investigated. However, more recently, the NIA-AA guidelines for AD and cognitive decline due to AD pathology introduced some evidence suggesting a direct relation between diet and changes in the brain structure and activity. Several studies focused on the role of the dietary patterns on late-life cognition, with accumulating evidence that higher adherence to a Mediterranean-type diet was associated with decreased cognitive decline, although the MeDi combines several foods, micronutrients, and macronutrients already separately proposed as potential protective factors against dementia and MCI. Moreover, also other emerging healthy dietary patterns such as the DASH and the MIND diets were associated with slower rates of cognitive decline and significant reduction in AD rate. Furthermore, some food groups traditionally considered harmful such as eggs and red meat have been partially rehabilitated, while there is still a negative correlation of cognitive functions with added sugars and trans fatty acids, nutrients also increasing the cardiovascular risk.

However, some limits should be reported for this review article. Heterogeneity exists in the quantification of individual items as well among the different diets background of the populations investigated, especially in view of different geographical areas, setting of dietary patterns such as the Mediterranean countries in which a large segment of the population still adheres to MeDi. Heterogeneity in time between the two assessments, among studies using paired assessments (or a single assessment) several years after study population enrollment. Nevertheless, these data represent a brick in the construction of the building of the causal link between dietary habits and cognitive impairment.

The absence of causal etiological therapies against AD leads to seek multimodal alternative strategies, increasing the interest in the potential for prevention of dementia by targeting modifiable risk factors. It is now evident that dietary habits influence diverse cardiometabolic risk factors, including not only obesity and low-density lipoprotein cholesterol, but also blood pressure, glucose-insulin homeostasis, lipoprotein concentrations and function, oxidative stress, inflammation, endothelial health, hepatic function, adipocyte metabolism, pathways of weight regulation, visceral adiposity, and the microbiome. Whereas decades of dietary recommendations focused on dietary fat and single vascular risk factors (e.g., hypertension, blood cholesterol etc.) and current dietary discussions are often worried about total calories and obesity, the full health impact of diet extends far beyond these pathways. Considering strategies of prevention of AD could be complicated and take to negative results. A second key lesson is the importance to point out on specific foods and overall diet patterns, rather than single isolated nutrients, for cognitive impairment. A food-based approach also better facilitates public guidance and minimizes industry manipulation. Nevertheless the complexity of the stake, the correction of modifiable risk factors to expect 'the compression of cognitive morbidity' still remains a desirable goal of public health. Larger observational studies with longer follow-up periods should be encouraged, addressing other potential bias and confounding sources, so hopefully opening new ways for diet-related prevention of dementia and AD.

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