

Steatosi Epatica non alcolica e da epatite C: Epidemiologia nutrizionale, Lifestyle medicine e Nutraceutica

Health&Wealth Unibs call 2015

Co-financed by: Professional Dietetics S.p.A; Calendoscopio ONLUS; Fondazione Iniziative Zooprofilattiche e Zootecniche; Università degli Studi del Molise

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1 Published paper

- **A New Self-Administered Semi-Quantitative Food Frequency Questionnaire to estimate nutrient intake among Italian Adults: Development Design and Validation Process.** *Nutrition Research*, 2020 Aug;80:18-27

3 manuscripts in preparation

8 Communications to national and international congresses

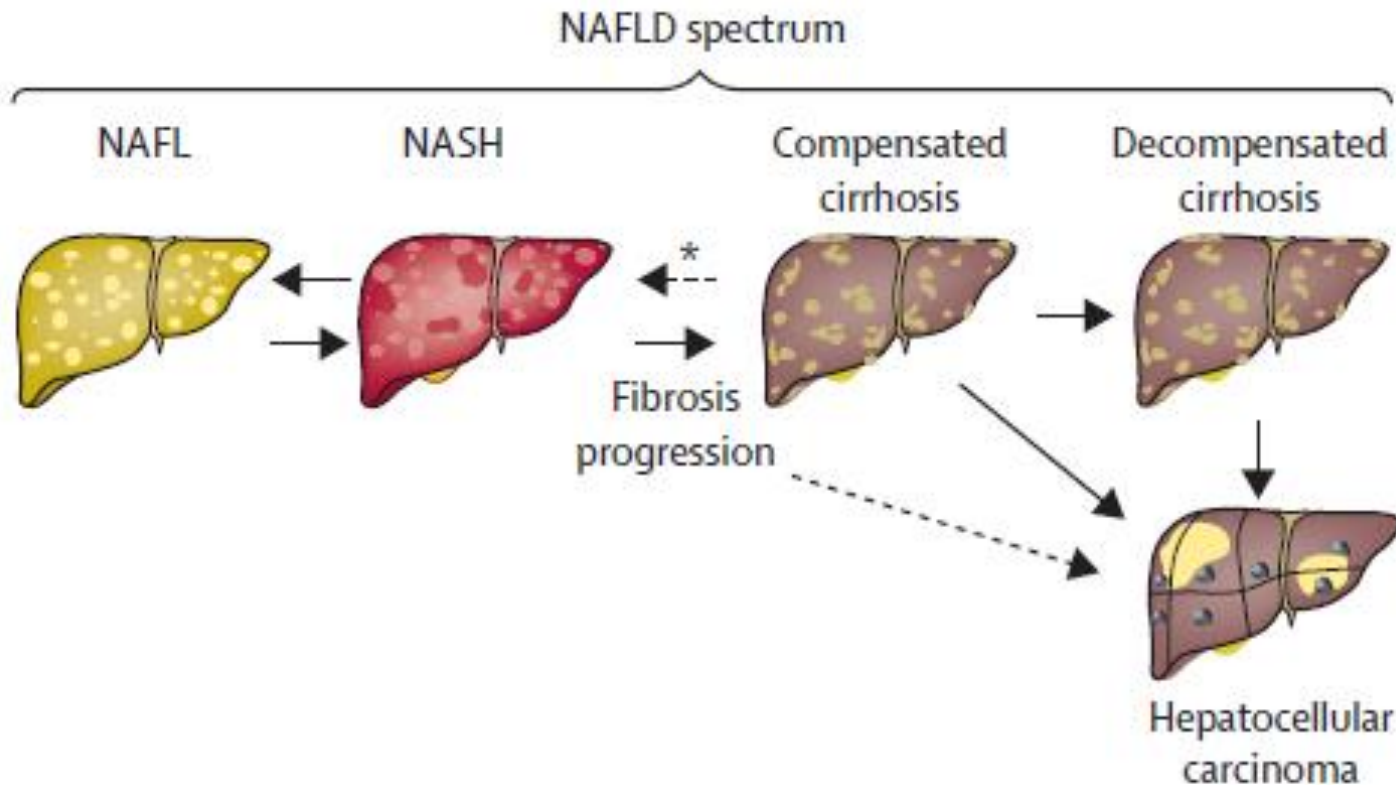
- **Disegno dello Studio e dati Preliminari del progetto SEELN: Steatosi Epatica non alcolica: Epidemiologia nutrizionale e Lifestyle Medicine.** *Poster Presentation, Congresso Nazionale, S.IT.I. - Società Italiana di Igiene, Medicina Preventiva e Sanita' Pubblica* 2017
- **Adherence to Mediterranean Diet and NAFLD diagnosis: preliminary results from an Italian prospective cohort study.** *Poster Presentation, IX Congresso Nazionale SIO, 11-13 Ottobre 2018, Milano, Italia.*
- **Dietary supplementation with a specific amino acid formula sustains mitochondrial biogenesis and reduces hepatic steatosis in a high-fat diet mouse model of non-alcoholic fatty liver disease.** *Poster Presentation, IX-Congresso Nazionale SIO, 11-13 Ottobre 2018, Milano, Italia.*
- **La supplementazione con aminoacidi ramificati promuove la biogenesi mitocondriale e riduce l'accumulo di grasso epatico in un modello murino di NAFLD.** *Comunicazione orale. Spazio Nutrizione, Milano, 22-23 marzo 2019*
- **Lifestyle modification in patients with Non-Alcoholic Fatty Liver Disease: results from an Italian single arm intervention study.** *Oral Communication, Nutrients Conference, 25-27 September 2019, Barcelona, Spain.*
- **Development and Validation of a Self-Administered Italian Semi-Quantitative Food Frequency Questionnaire to Estimate Nutrient Intake.** *Oral Communication, Nutrients Conference, 25-27 September 2019, Barcelona, Spain.*
- **Patients with Non-Alcoholic Fatty Liver Disease and lifestyle modification: results from an Italian Mediterranean oriented intervention study.** *Oral Communication II European Lifestyle Medicine Organization Congress, 8-10 November 2019, Rome, Italy.*
- **Dietary supplementation with a specific amino acid formula sustains mitochondrial biogenesis and reduces hepatic steatosis in a high-fat diet mouse model of non-alcoholic fatty liver disease.** *Poster Presentation, 39° Congresso Nazionale della Società Italiana di Farmacologia (SIF), Firenze, 20-23 Novembre 2019*

3 Tesi di laurea Corso di Studio di Medicina e Chirurgia

4 Tesi di laurea Corso di Studio in Dietistica

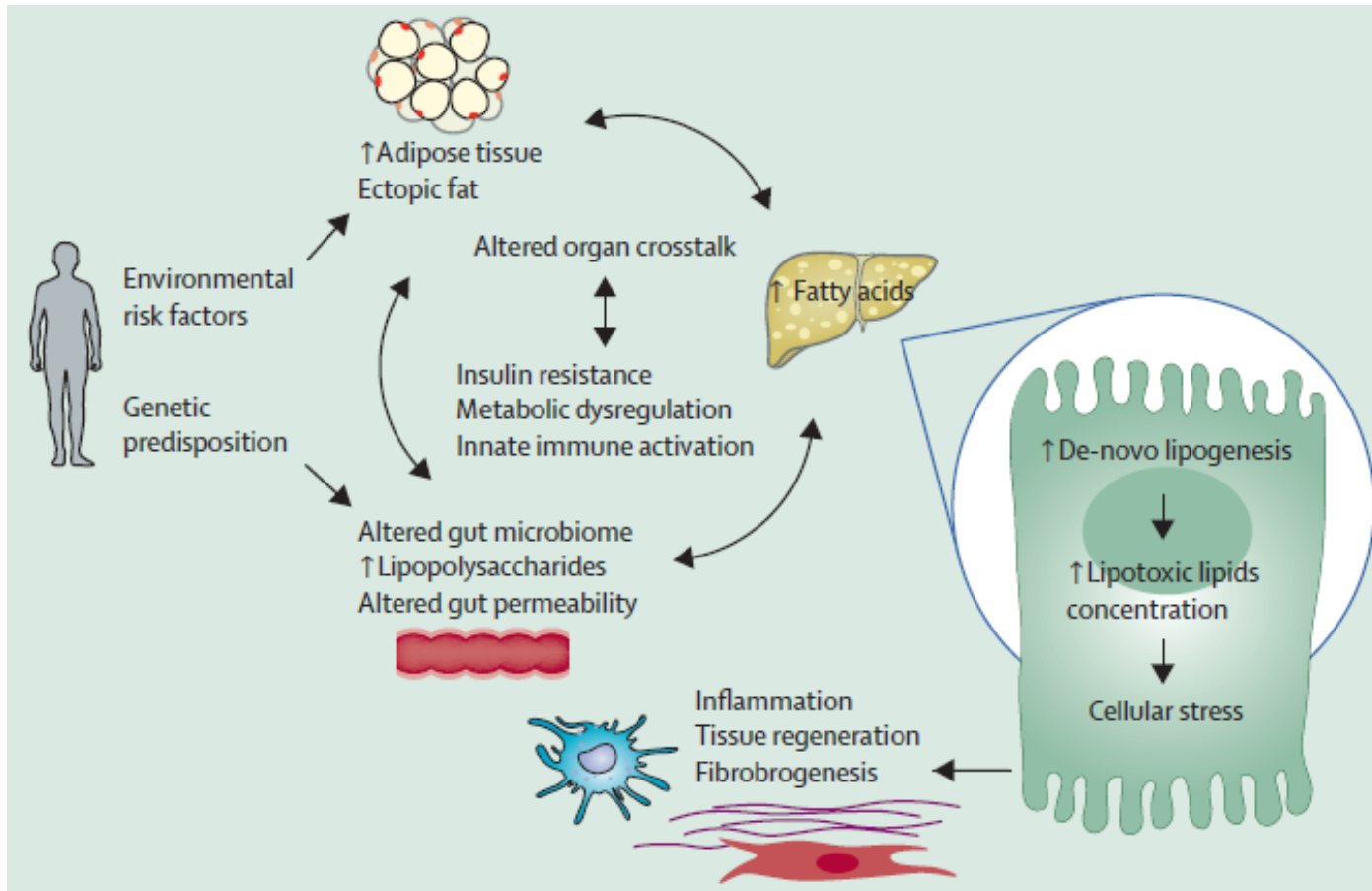
- **Italian Food Frequencies Questionnaires (Seeln-FFQ)**

NAFLD refers to a group of conditions where there is accumulation of excess fat in the liver



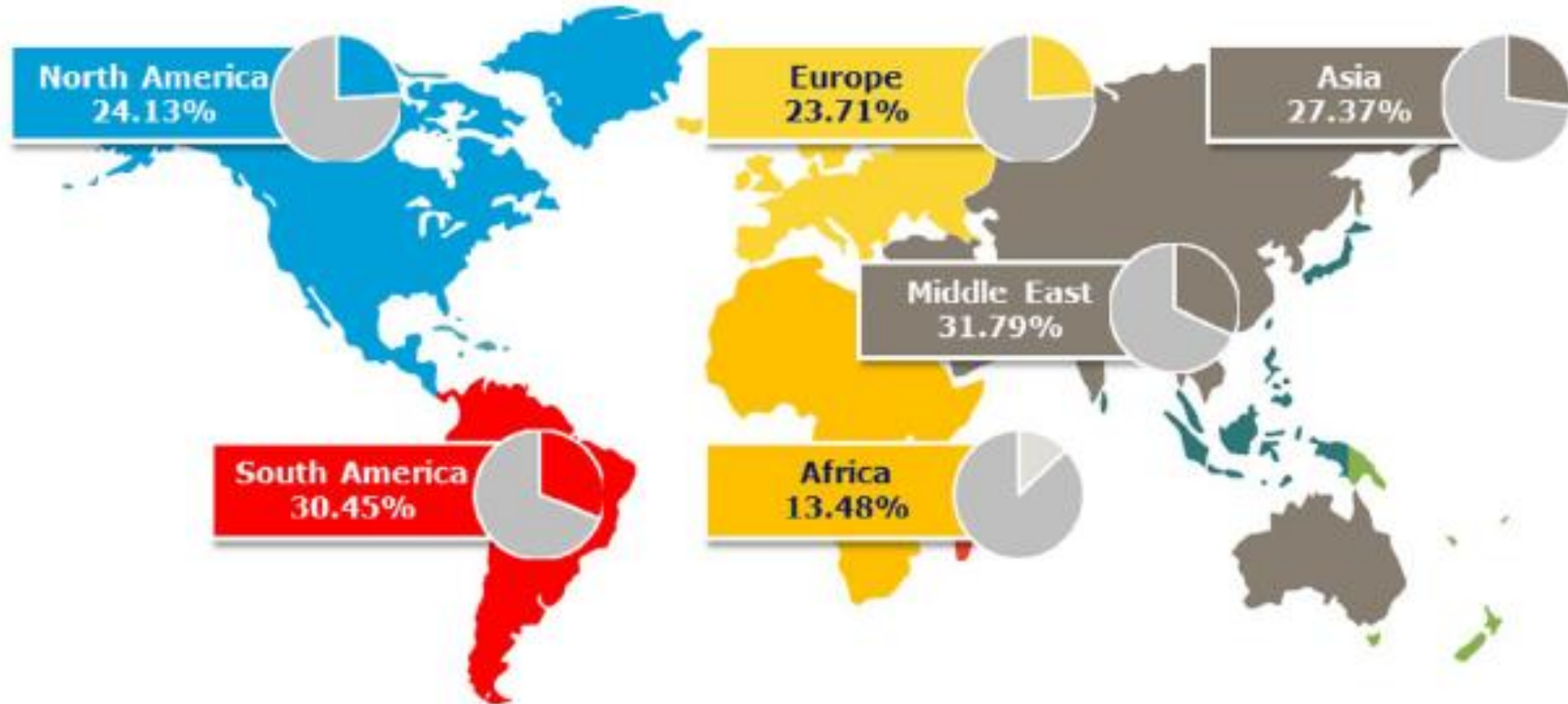
- NAFLD encompasses a disease continuum from liver steatosis to non-alcoholic steatohepatitis (NASH) with inflammation and fibrosis progression.
- It is an important cause of end-stage liver disease, primary liver cancer, and liver transplantation
- Advanced liver fibrosis is a key prognostic marker for liver-related outcomes and overall mortality

NAFLD pathogenesis and pathways involved



- Both **genetic and environmental risk factors** contribute to NAFLD
- Multiple pathways and **interactions among different organs** affect NAFLD pathogenesis
- NAFLD is considered as the **hepatic component of the metabolic syndrome**, with **insulin resistance** as the key factor in the pathophysiology

Global prevalence of Non Alcoholic Fatty Liver Disease (NAFLD)



Main challenges in NAFLD management:

- NAFLD is largely **under-recognised** by health-care professionals and the wider community
 - Lack of a **reliable biomarker** to diagnose and stage NAFLD across the entire disease spectrum
 - Substantial **heterogeneity** of NAFLD and the current limited understanding of **disease phenotypes**
 - There is currently **no approved therapy** for NAFLD
- ❖ As obesity is the main driver of this common liver disease and its associated metabolic comorbidities, **healthy lifestyle and weight reduction remains crucial** for the prevention and treatment of NAFLD

Aims of the SEELN project

✓ ***Nutritional Epidemiology***

- to develop and validate a new Italian Food frequency Questionnaire
- to evaluate the relationships between diet and NAFLD

✓ ***Lifestyle medicine (clinical trial)***

- to assess the efficacy of a Mediterranean-oriented lifestyle modification algorithm for patients with NAFLD (single arm intervention study)
- substudy to identify novel disease biomarkers

✓ ***Nutraceuticals (preclinical study)***

- to assess the role of mitochondrial impairment in NAFLD pathogenesis
- to explore the protective role of dietary supplements

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✓ **Nutritional Epidemiology**

To develop and validate a new culture-specific semi-quantitative Italian Food frequency Questionnaire (FFQ) for **self-administration** adapting a previously validated FFQ (Fred Hutchinson Cancer Research Center)

- The new target population
- Cultural background
- Local dietary practices
- New dietary trends

The **SEELN FFQ** consisted of:

- **5 general questions about food choices** (cooking methods of meat, poultry, eggs, type of pasta or pizza generally consumed)
- **145 food items** divided in 9 sections as follow: “grains, bread and snacks”, “first or main course”, “meat, fish and eggs”, “dairy products”, “vegetables and pulses”, “sauces and dressing”, “fruits”, “desserts”, “beverages and drinks”.

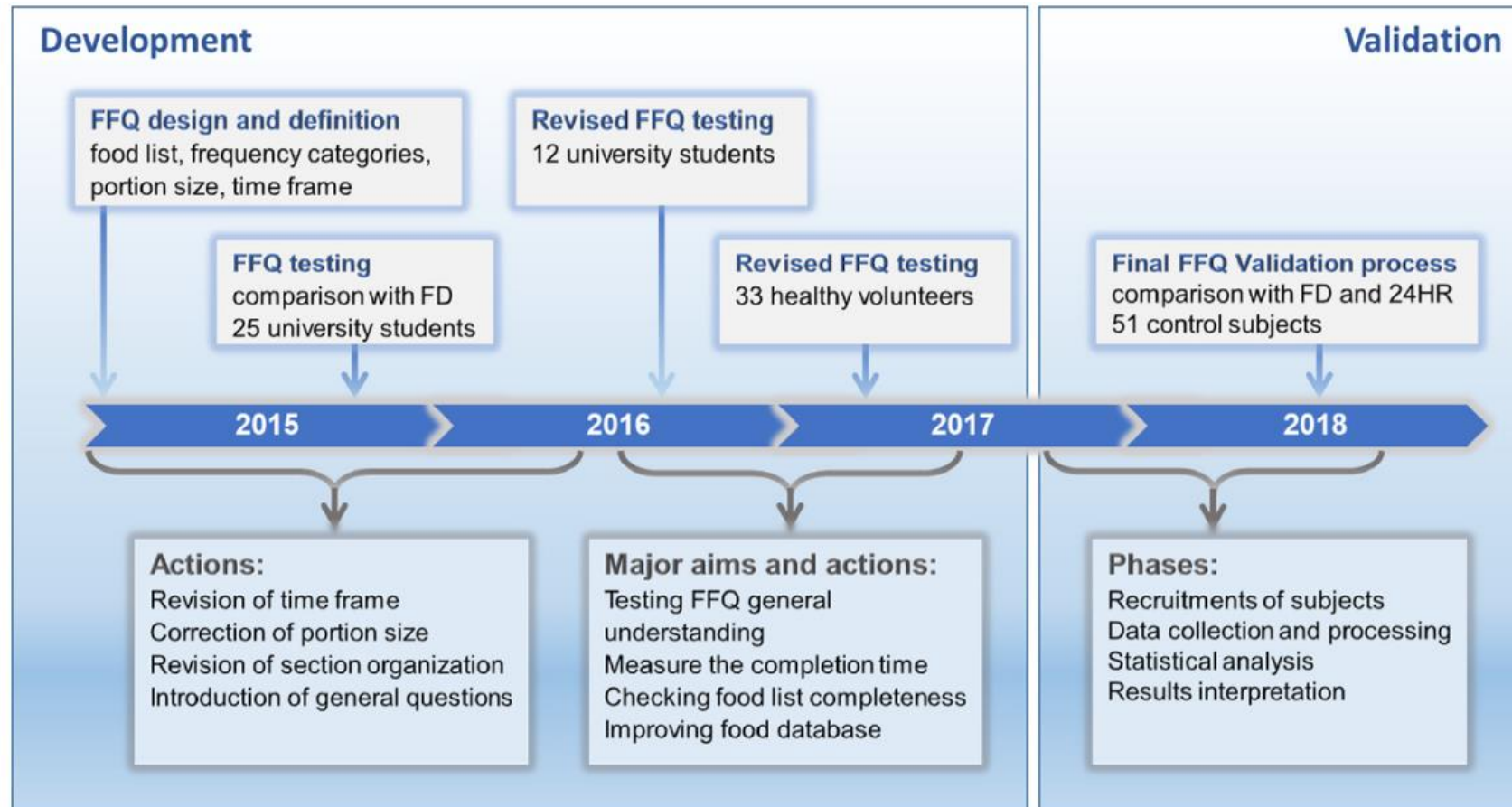
Section sample of FFQ with food frequencies and quantities

CEREALI PER LA COLAZIONE, PANE, SNACKS													
	Con che frequenza ha consumato questi cibi?									In che quantità?			
	Mai o meno di 1 al mese	1-3 volte al mese	1 volta alla sett.	2-4 volte alla sett.	5-6 volte alla sett.	1 volta al giorno	2-3 volte al giorno	4-5 volte al giorno	6 o più volte al giorno	Dimensioni di una porzione media	S	M	L
Cereali (es. fiocchi di mais/avena, crusca, cereali, cereali soffiati, gallette)										3-4 cucchiari o 2 gallette			
Muesli										3 cucchiari			
Fette biscottate										3 fette			
Biscotti secchi										5 pezzi			
Biscotti trollini										3 pezzi			
Brioche, muffin, croissant										1 pezzo			
Merendine confezionate										1 pezzo			
Pane bianco										1 panino (da 1 pugno) o 2 fette			

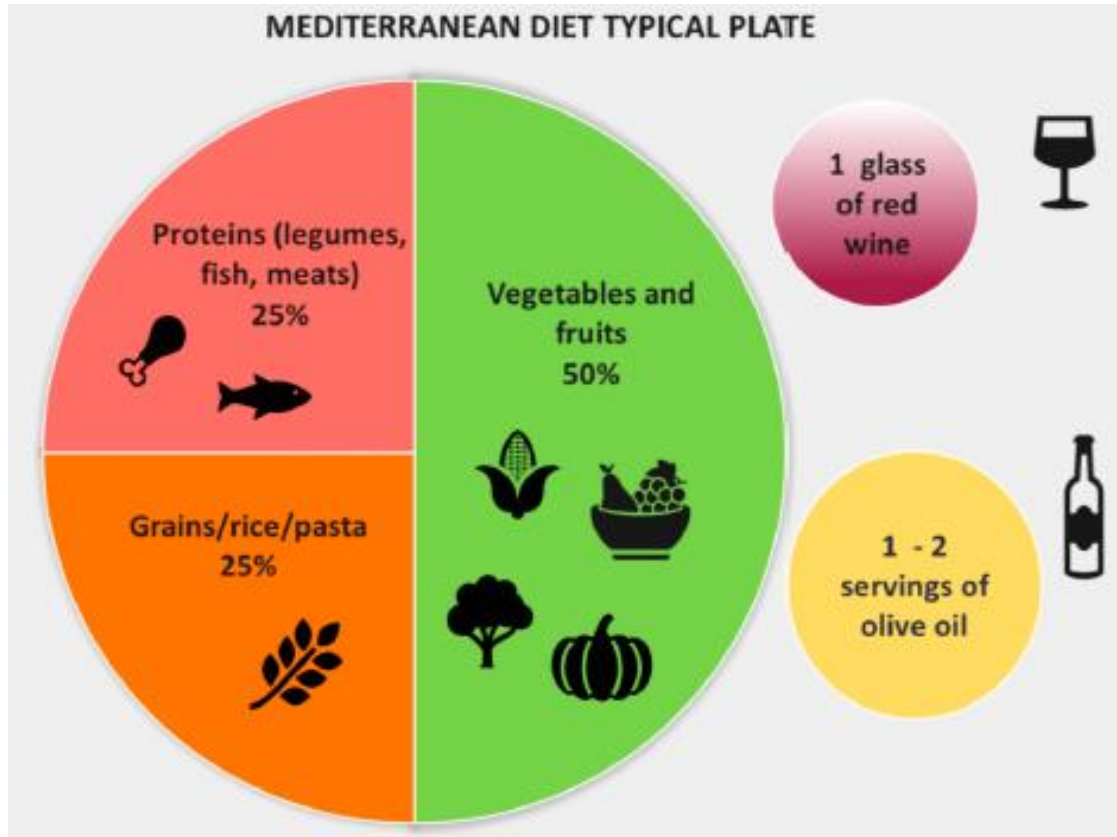
A new self-administered semi-quantitative food frequency questionnaire to estimate nutrient intake among Italian adults: development design and validation process



Barbara Zanini^{a,*}, Anna Simonetto^b, Paola Bertolotti^b, Monica Marullo^c, Silvia Marconi^b, Chiara Becchetti^a, Gianni Gilioli^b, Alessandra Valerio^b, Francesco Donato^c, Chiara Ricci^{a,d}, Maurizio Castellano^{a,d}



NAFLD: Lifestyle intervention and Mediterranean diet



ADHERENCE to Mediterranean Diet

Among the 145 food items of the SEELN FFQ, we identified those presumed to be closer to Mediterranean Diet (MD) and assigned a score according to their frequency consumption (**SeeInMedScore**)

Higher values of the score indicated greater adherence to MD

Food	Frequency, assigned score		SeeInMedScore (0-25)	
Whole pasta	<50%, 0		50-100%, 1	
Whole bread	Less than once per month to once per week, 0	2-4 times per week to 5-6 times per week, 1		At least 1 time per day, 2
Vegetables	Less than 5-6 times per week, 0	Once per day, 1	2-3 times per day, 2	At least 4-5 times per day, 3
Fruits	Less than 2-4 times per week, 0	5-6 times per week, 1	once per day, 2	At least 2-3 times per day, 3
Pulses	Less than 1-3 per month, 0		once per week, 1	At least 2-4 times per week, 2
Fish	Less than 1-3 per month, 0		once per week, 1	At least 2-4 times per week, 2
Nuts	Less than once per week, 0		2-4 times per week to 5-6 times per week, 1	At least 1 time per day, 2
Olive Oil	Less than 5-6 times per week, 0		Once per day, 1	At least 2-3 times per day, 2
Wine	Less than 5-6 times per week or more than 2-3 times per day, 0			Once per day, 1
Processed Meat	Less than once per month, 1			At least 2-3 times per month, 0
Saturated Fat	Less than 5-6 times per week, 0			At least 2-3 times per week, 1

Clinical trial:

Non Alcoholic Fatty Liver Disease: Nutritional Epidemiology and Lifestyle Medicine

ClinicalTrials.gov Identifier: NCT03300661

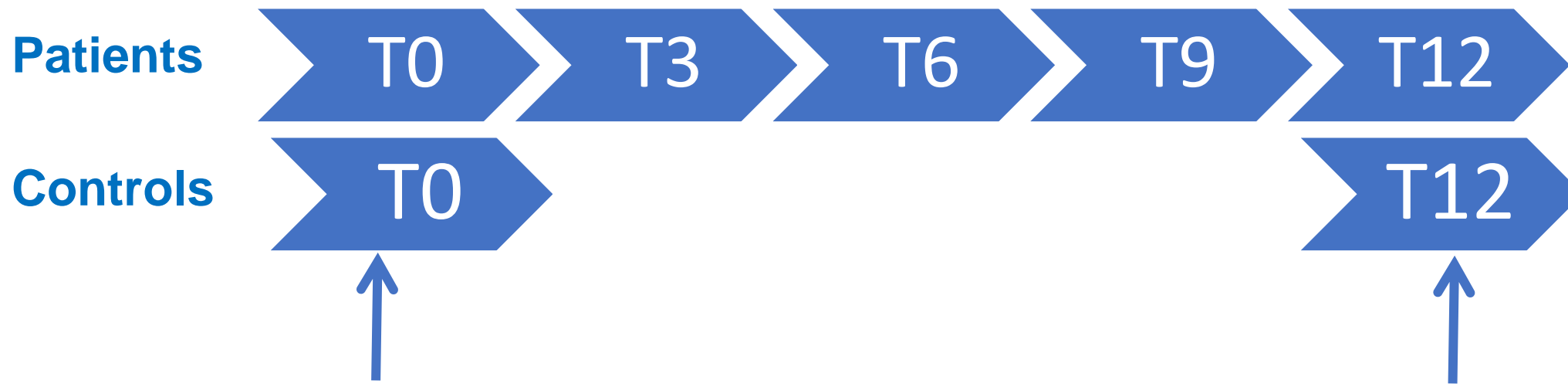
Sigla Protocollo: SEELN 2016

Data di inizio 01/03/2017

Data termine: 28/02/2019 → 28/02/2020

SEELN clinical trial: methods

- Selection criteria: males and females, aged 18-60 years, with no severe clinical condition and with no other liver damage reason
- According to a liver ultrasound, selected subjects were divided in:
 - Patients' group (with mild, moderate or severe steatosis)
 - Healthy controls' group (no steatosis)
- All participants
 - Clinical evaluation
 - Laboratory blood test
 - Liver-ultrasound
 - BMI (Height, weight) + waist circumference
- Data on:
Educational level, occupation, smoking habits, alcohol intake, ongoing treatment, present and past co-morbidities



Patients

T0

T3

T6

T9

T12

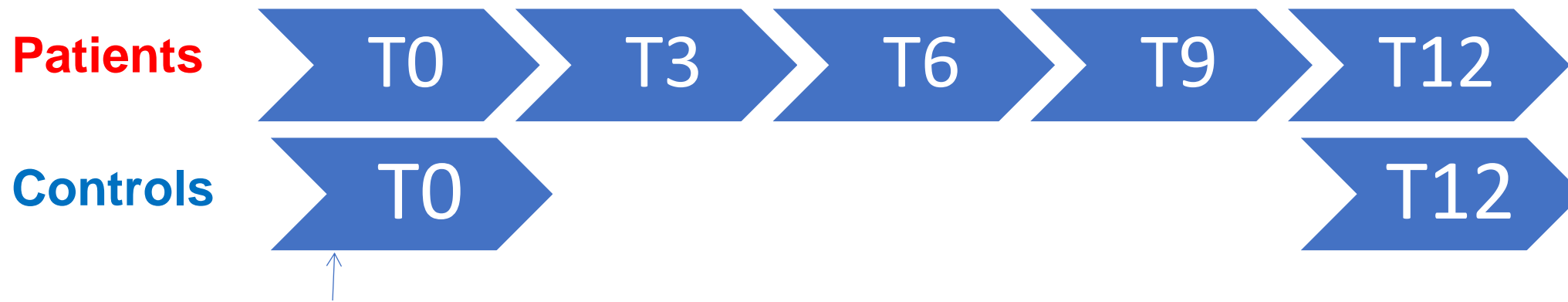
Controls

T0

T12

- International Physical Activity Questionnaire (IPAQ)
- FFQ+ SEELN-MedScore
- Clinical evaluation
- Laboratory blood test
- Liver-ultrasound
- BMI (Height, weight) + waist circumference

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- **International Physical Activity Questionnaire (IPAQ)**
- **FFQ + SEELN-MedScore**

Patients

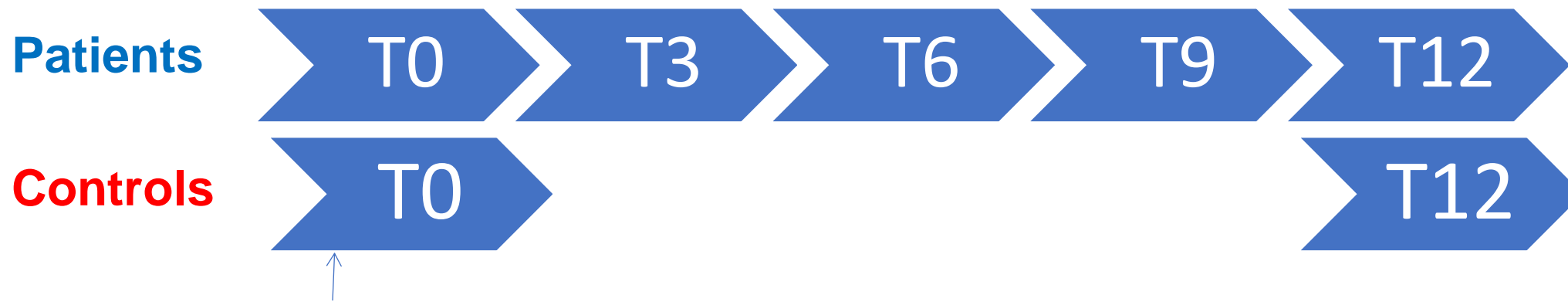
T0

3 personalized dietary Mediterranean-oriented and 1 physical activity advices were provided and written in a patient booklet;

3 simple cooking recipes, according to dietary advices, were printed and delivered

T3, T6, T9

The same procedures were offered by two registered dieticians in order to improve and reinforce adherence.

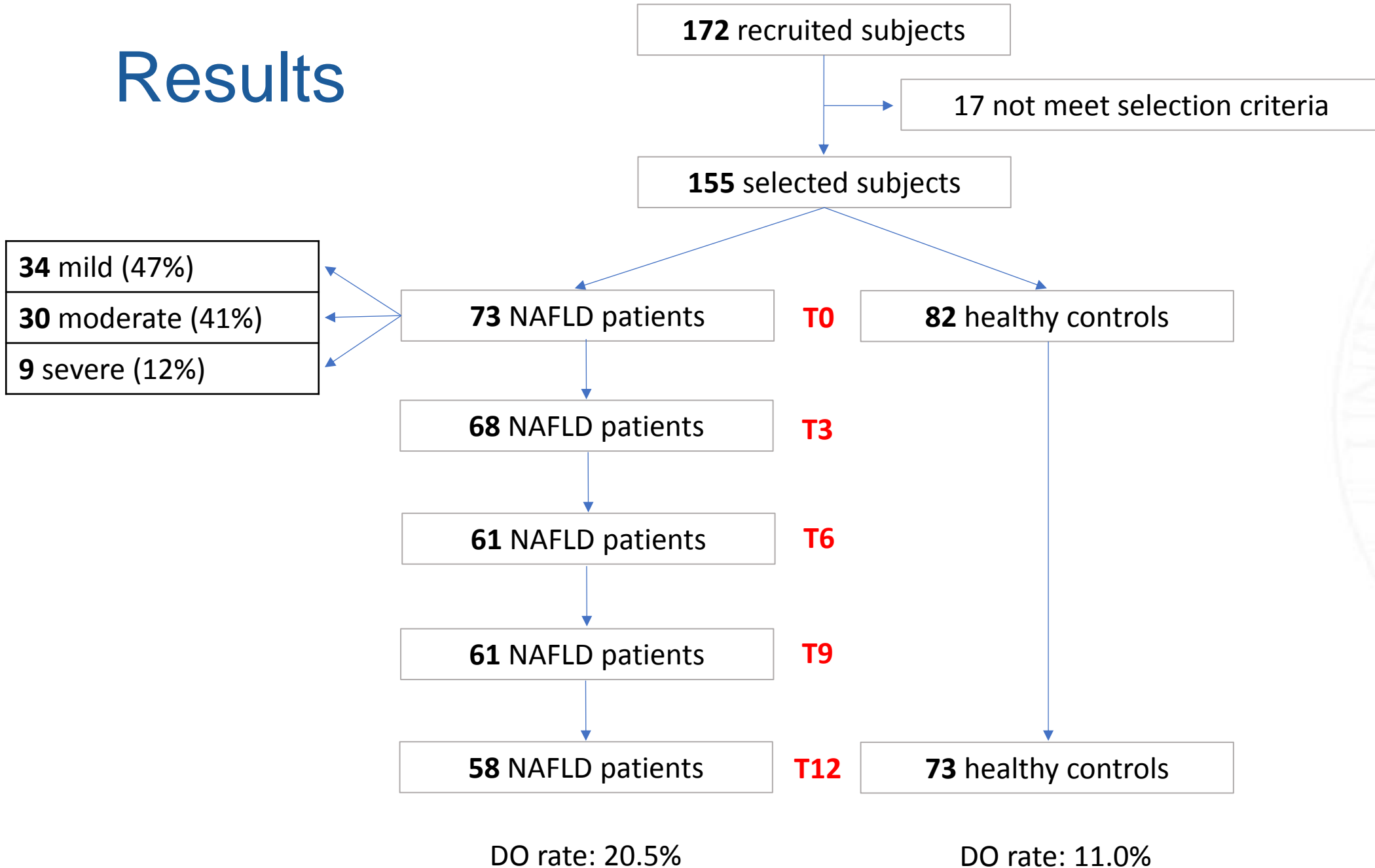


- International Physical Activity Questionnaire (IPAQ)
- FFQ + SEELN-MedScore

Controls

T0 Mediterranean Diet was presented, a complete explanation of the key aspects of the diet were discussed and a simple booklet was provided

Results

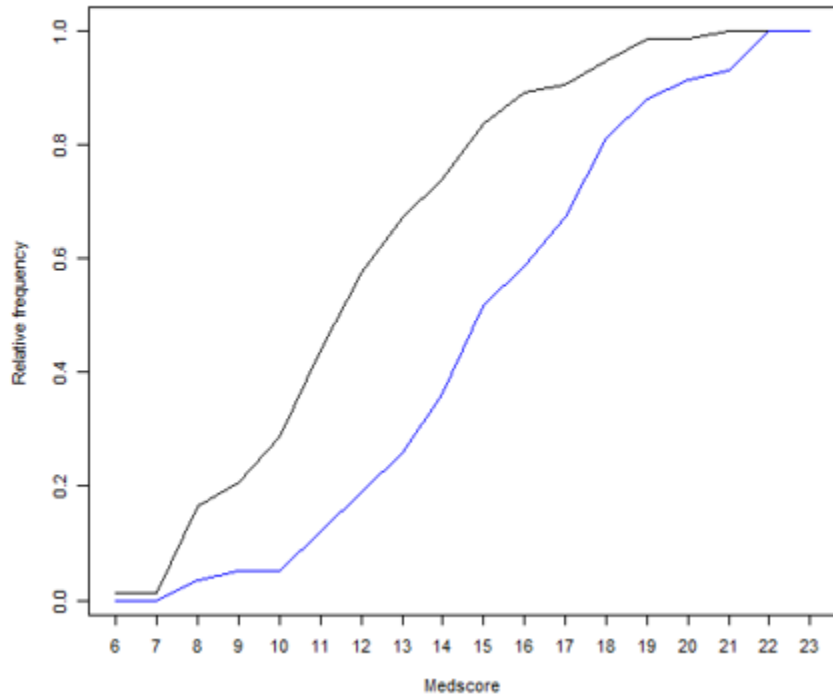


	T0			T12		
	Median		Mann-Whitney	Median		Mann-Whitney
	Patients (73)	Controls (82)	p-value	Patients (58)	Controls (73)	p-value
Weight (kg)	89.5	65.0	<0.0001	85.3	65.9	<0.0001
BMI	31.3	23.8	<0.0001	30.5	24.0	<0.0001
WC (cm)	103	85.5	<0.0001	103	87	<0.0001
Col_tot (mg/dL)	207	191	0.02371	211	201	<i>0.2352</i>
HDL(mg/dL)	49	64	<0.0001	50	66	<0.0001
LDL (mg/dL)	126	111	0.00947	129	119	0.0081
Tg (mg/dL)	126	68	<0.0001	116	77	<0.0001
Glycemia (mg/dL)	93	83	<0.0001	95	84	<0.0001
Insulin (mU/L)	12	4	<0.0001	12	4	<0.0001
AST (U/L)	21	18	0.01435	21	19	<i>0.8671</i>
ALT (U/L)	38	23	<0.0001	34	24	<0.0001
Homa	2.9	0.8	<0.0001	2.9	0.8	<0.0001
MET	1800	2195	0.0711	1865	1518	0.5705
Medscore	12	15	<0.0001	15	16	<i>0.7422</i>

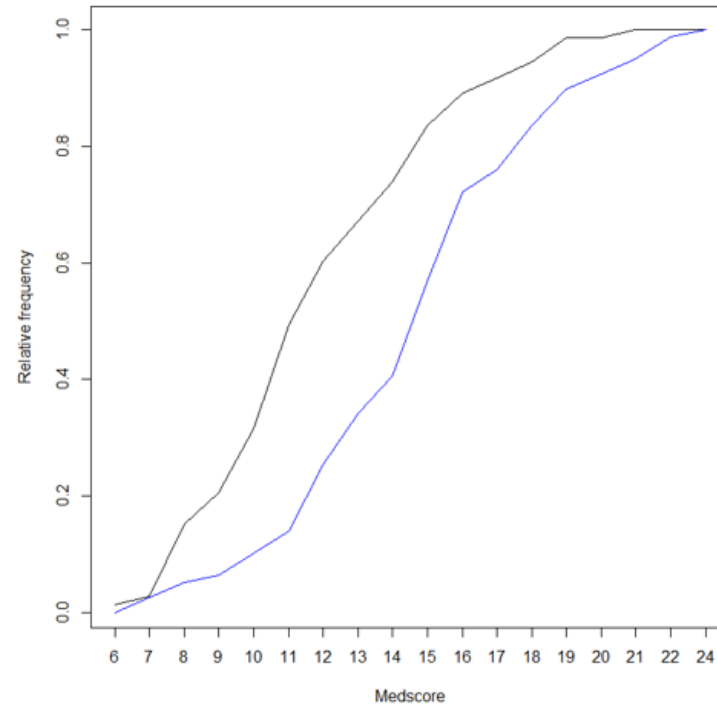
	Patients			Controls		
	Median		Wilcoxon test	Median		Wilcoxon test
	T0 (73)	T12 (58)	p-value	T0 (82)	T12 (73)	p-value
Weight (kg)	89.5	85.3	<0.0001	65	65.9	0.9873
BMI	31.3	30.5	<0.0001	23.8	24.0	0.9771
WC (cm)	103	103	0.0755	85.5	87	0.02708
Col_tot (mg/dL)	207	211	0.2928	191	201	0.06037
HDL(mg/dL)	49	50	0.9958	64	66	0.4351
LDL (mg/dL)	126	129	0.2201	111	119	0.08964
Tg (mg/dL)	126	116	0.02924	68	77	0.5796
Glycemia (mg/dL)	93	95	0.5576	83	83.5	0.5314
Insulin (mU/L)	12	12	0.1003	4	4	0.7335
AST (U/L)	21	20.5	0.748	18	20	0.02792
ALT (U/L)	38	34	0.4308	23	24	0.3735
Homa	2.9	2.9	0.2304	0.8	0.8	0.8861
MET	1800	1865	0.9166	2195	1518	0.0059
Medscore	12	15	<0.0001	15	16	0.1723

Focus on SEELN-MedScore

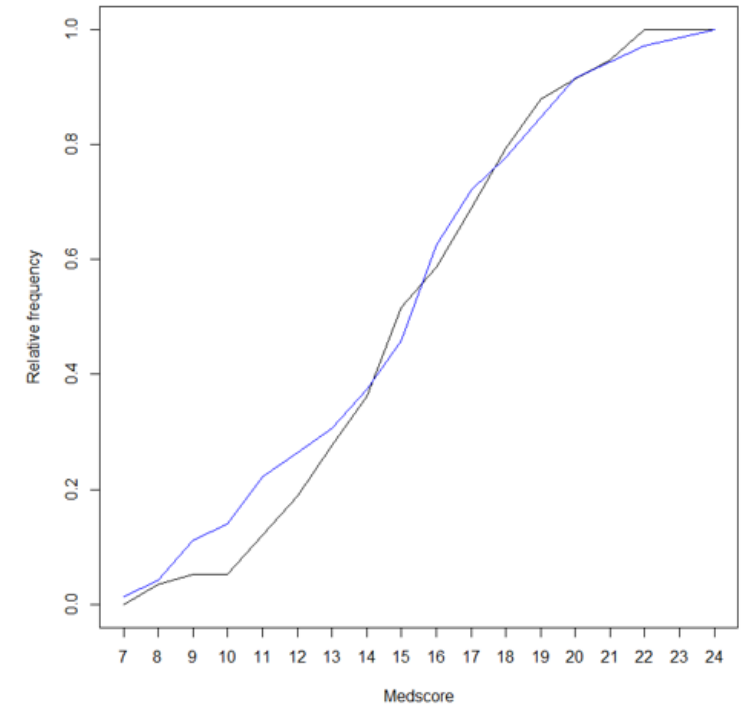
Patients - Comparison Month 0 (black) and 12 (blue)



Comparison Patient (black) - Control (blue) at Month 0



Comparison Patient (black) - Control (blue) at Month 12



Main endpoint

12 patients with complete remission (21% PP, 16%ITT)

14 patients with regression of steatosis (24% PP, 19% ITT)

Steatosis degree	T0	T12
No	0	12 (21%)
Mild	34 (42%)	16 (28%)
Moderate	30 (41%)	22 (38%)
Severe	9 (12%)	7 (12%)

	T0			T12		
	Median		Wilcoxon test	Median		Wilcoxon test
	Not improved	Improved	p-value	Not improved	Improved	p-value
N	32	26		32	26	
Weight (kg)	90.3	87.6	0.5063	85.3	84	0.3563
BMI	31.4	29.6	<i>0.0746</i>	30.7	28.0	<i>0.0667</i>
WC (cm)	104	101	0.3144	104	101	0.2567
Col_tot (mg/dL)	208	207	0.5597	202	220	0.6367
HDL(mg/dL)	46	51	0.0143	50	56	0.0332
LDL (mg/dL)	127	125	0.386	125	140	0.9149
Tg (mg/dL)	149	113	0.0387	132	106	0.2058
Glycemia (mg/dL)	93	93	0.8857	92.5	100	0.2536
Insulin (mU/L)	16	9	0.0018	14	10	0.0149
AST (U/L)	20	21.5	0.6694	21	19.5	0.3976
ALT (U/L)	46	34	0.0504	40.5	34	0.5094
Homa	3.57	1.98	0.0076	3.267	2.173	0.0398
MET	1800	2460	0.3432	1830	2312.5	0.5523
Medscore	11	13	0.6203	16	15	0.5248

Logistic regression

	Median Var %
Col_tot	1.51 %
HDL	4.31 %
LDL	3.42 %
Tg	-9.60 %
Glycemia	0.43 %
Insulin	5.26 %
AST	0 %
ALT	-4.48 %
Homa	-2.93 %
MET	1.01 %
Weight	-2.39 %
Medscore	26%

Variable	Regression coefficient estimate	St.error	Z value	p-value
Intercept	-0.6561	0.5002	-1.312	0.1896
Var_Tg	1.7115	1.0257	1.669	0.0952
Var_MET	-0.2641	0.1943	-1.359	0.1741
Var_Weight Dicotomic (3%)	1.5536	0.7568	2.053	0.0401

Conclusions

- The rates of improvement of steatosis were consistent with previous lifestyle intervention studies on NAFLD
- Weight reduction of at least 3% was strongly associated with the main outcome
- Strategies to improve the adherence (DO rate of 21%)
- Maintenance in the long period

Aims of the SEELN project

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- to develop and validate a new Italian Food frequency Questionnaire
- to evaluate the relationships between diet and NAFLD

✓ ***Lifestyle medicine (clinical trial)***

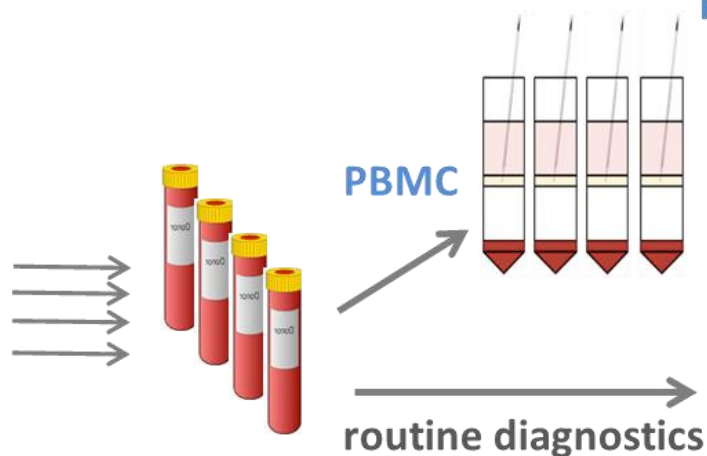
- to assess the efficacy of a Mediterranean-oriented lifestyle modification algorithm for patients with NAFLD (single arm intervention study)
- **substudy to identify novel disease biomarkers**

✓ ***Nutraceuticals (preclinical study)***

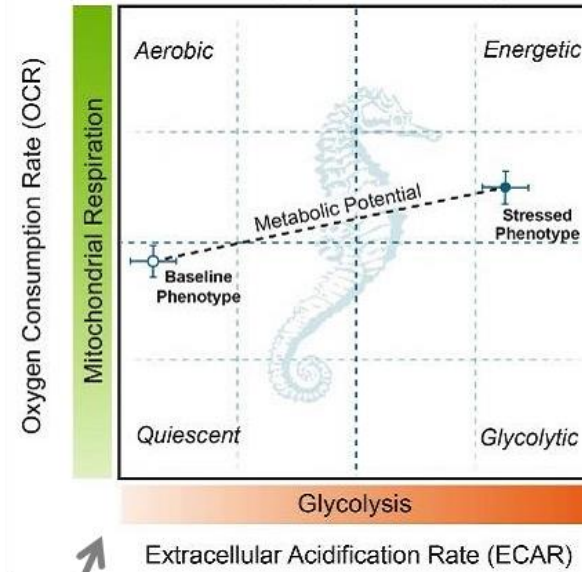
- to assess the role of mitochondrial impairment in NAFLD pathogenesis
- to explore the protective role of dietary supplements

Substudy to identify novel NAFLD biomarkers

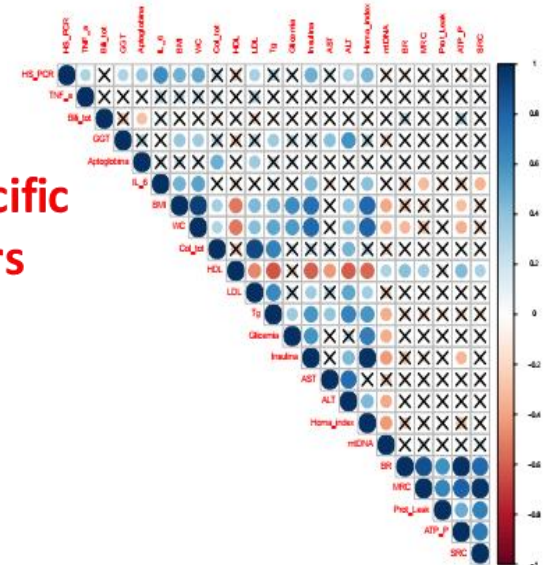
- Mitochondrial bioenergetics in peripheral blood mononuclear cells (PBMCs)
- Circulating cytokines
- Ultrasound assessment and other routine analyses



metabolic phenotype



panel of disease-specific biomarkers



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Dietary supplementation with a peculiar amino acid formula sustains mitochondrial bioenergetics and reduces hepatic damage in a mouse model of non-alcoholic fatty liver disease

- Diet-induced NAFLD in adult male C57BL/6J mice (n = 12/ group)
 - Standard diet
 - Standard diet + $\alpha 5$
 - High-fat high-sugar diet (HFHSD)
 - High-fat high-sugar diet + $\alpha 5$
- ❖ Feeding mice with **high-fat high-sugar diet (HFHSD)** increased body weight and **increased NAFLD Activity Score in mouse liver**
- ❖ Dietary supplementation with **$\alpha 5$ significantly reduced the NAFLD Activity Score** and hepatic lipid droplets diameter in HFHSD-fed mice
- ❖ Dietary supplementation with **$\alpha 5$ increases liver mitochondrial DNA content** in HFHSD-fed mice
- A clinical trial to investigate the effects of $\alpha 5$ in NAFLD patients is planned

Essential amino acids	$\alpha 5$
L-Leucine	31.09
L-Lysine (chlorhydrate)	16.90
L-Isoleucine	10.36
L-Valine	10.36
L-Threonine	7.25
L-Cysteine	3.11
L-Histidine	3.11
L-Phenylalanine	2.07
L-Methionine	1.04
L-Tyrosine	0.62
L-Tryptophan	2.07
Vitamin B1 (thiamine chlorhydrate)	0.004
Vitamin B6 (pyridoxine chlorhydrate)	0.004
Citric acid	8.00
Malic acid	2.00
Succinic acid	2.00
Leucine : isoleucine : valine ratio	3 : 1 : 1

Ruocco et al, Curr Opin Clin Nutr Metab Care, 2021