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Improving Adherence to Mediterranean Diet to prevent or reduce Metabolic Syndrome in Heart Transplanted Patients

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Abstract

The Metabolic Syndrome (MetS) is a multi-factorial condition, which enhances the risk to develop chronic related diseases, such as cardiovascular diseases, cancer and neurological disorders. Despite many advances in patients' management and pharmacological treatment, MetS represents a real burden in heart transplanted patients, mainly due to the side effects of immunosuppressive therapy which severely affects their long-term outcomes. The improvement of dietary habits seems to represent an effective strategy to reduce the MetS in general population, decreasing the cardiovascular risk factors. Among all, number of studies associate the Mediterranean diet to a reduction of cardiovascular incidence and of all-cause mortality. One of the main beneficial effect of this dietary pattern is the anti-inflammatory action, which could be of great importance in heart transplanted patients. Unfortunately, dietary programs for the long-term period after heart transplantation are not yet adequately provided in the routine follow-up of these patients.

The **first Aim** of this PhD research was to assess the prevalence of MetS in heart transplanted patients at the University Hospital of Udine, since 2007, and evaluate the impact on the long-term outcome in terms of morbidity and mortality. Through a retrospective collection of clinical data, we observed that more than half (52%) of the patients were affected by MetS at 5 years of follow-up and that the early development of this condition, both before and within 1 year of transplantation, were associated to a worst survival and to a higher risk to develop cardiac allograft vasculopathy.

Since the beneficial effects of Mediterranean diet on cardiovascular risk factors, the **second Aim** of the project was to assess the adherence to the Mediterranean diet of 143 cardiac transplanted patients at the University Hospital of Udine. Through the administration of a validated Food Frequency Questionnaire (FFQ) we observed an overall weak adherence to the Mediterranean diet, together with an inadequate consumption of many healthy foods characteristic of this dietary pattern.

Finally, the **Aim 3** was to evaluate any beneficial effect of a structured and personalized dietary intervention in a sample of cardiac transplanted patients. Changes were compared at baseline and at each timepoint, within the intervention group and between the intervention and the control group, which followed general nutritional advices. The variables of interest were the adherence to the Mediterranean diet, clinical, anthropometric, body composition and blood parameters data, and general dietary habits. Fifteen patients were recruited for the intervention group versus 13 in the control group. The comparison between intervention and control group occurred at baseline and at the intermediate meeting, after 6 months, reached by 11 patients of the intervention group versus and

10 patients of the control group. The analysis within the intervention group included also the final meeting, after 12 months, reached by 7 patients. The main findings were:

1) Mediterranean diet adherence significantly increased in the intervention group during the study period, both comparing the intervention and the control group, and within the intervention group over the three timepoints;

2) moreover, parameters of body composition in the intervention group significantly changed over the study period, with a significant decrease of fat mass % and a subsequent increase of fat free mass %, and body cell mass %; also waist circumference decreased on average of -1.3±2.6cm. Furthermore, both blood pressure and renal function resulted significantly improved in the intervention group versus the control group, at the intermediate timepoint.

3) finally, dietary habits of the intervention group showed an improvement of macronutrients balance, a significant decrease of energy from saturated fatty acids and soluble sugars, and a positive trend to micronutrients intake, over the study period.

Concluding, we confirmed that the implementation of a structured and personalized dietary programme may be feasible in heart transplanted population and exerts many beneficial effects. The future goal will be to offer a nutritional education, in line with the Mediterranean diet principles, to all cardiac transplanted patients at the University Hospital of Udine, with the intent to insert this treatment as a routine prescription in the standard follow-up of these patients.

1 Background

1.1 Heart transplantation: the achievements and the present challenges

More than 50 years have passed since the very first human heart transplantation, performed by Dr. Christiaan Barnard, on December 3, 1967. “I believe we’ve given the first step, and there’s still a long journey ahead but at least we have hope of completing this journey”, said Dr. Barnard during an interview.

As a matter of fact, since that first step, every aspect of this complex surgery, from the organ preservation to the immunosuppressive treatment and to recipients’ management during follow-up, has achieved continued advances in the last half century. All these achievements resulted in a considerably improvement in recipients’ survival, reaching a median survival of ≥ 12 years in the last two decades, as described in the most recent report by the International Society of Heart and Lung Transplantation (ISHLT) (1).

Heart transplantation is considered the gold standard therapeutic option in end-stage heart failure patients, when the disease evolves in a form resistant to any pharmacological treatment. According to the Global Burden of Diseases, Injuries, and Risk Factors Study 2017, an estimated 64.3 million people are living with heart failure worldwide (2), a number destined continuously to rise, due to a growing and ageing population. Consequently, the annual number of cardiac transplantations worldwide is increasing, with >5500 heart transplants performed in 2017, as well as the proportion of patients aged >60 years old receiving heart transplant (3).

An important point to consider is that the main goal of cardiac transplantation is not just to prolong recipient’s life, but rather to allow them to regain a favourable quality of life and active lifestyle after a long period of disease and illness, consequent to heart failure. However, the heart transplanted subject is a very complex patient, often affected by many comorbidities already present before surgery, with a lifelong immunosuppressive therapy that commonly predisposes them to other complications during the follow-up. These patients need to be constantly monitored for the rest of their life, in order to preserve optimal graft function and minimize the other complications.

Any strategy to support the physicians in preventing or mitigating adverse events in the long-term of these patients would be of paramount importance.

Usually, this strict follow-up is mainly centred on pharmacological treatment, adjusting or adding therapy in order to control the development of complications and cardiovascular risk factors. However, diet and nutrition are quickly emerging in the battle against chronic inflammatory diseases

and the potential to extend this front into the field of transplantation is rapidly taking root. Chronic diseases like obesity, diabetes, hyperlipidaemia and hypertension pose a significant long-term health burden for solid organ transplant recipients. Because nutritional status is a potentially modifiable risk factor, the development of strategies designed to optimize nutritional status decreases the risk of developing complications in the post-transplant period. In the following paragraphs an overview of the most common complications related to heart transplantation and immunosuppressive therapy will be provide, as long as a potential effective strategy to improve long-term outcome in cardiac transplanted patients, acting on a very common modifiable factor: the dietary habits.

1.2 Immunosuppressive therapy

As mentioned above, heart transplanted patients must follow a lifelong immunosuppressive therapy. The host immune response against the allograft is a physiologic human body's ability to recognize genetically dissimilar tissue and inducing an appropriate immune response. In this specific case, the immunological activation must be controlled in order to avoid the rejection of the transplanted graft. Since rejection can occur at any time over the life of the allograft, the immunosuppression starts at surgery and continues for the entire life of the patient, striking a delicate balance between modulating the immune system enough to prevent rejection while avoiding the adverse effects of immunodeficiency (3) and non-immune toxicities (nephrotoxicity, hypertension, hyperglycaemia, hyperlipidaemia) (Fig. 1) (4).

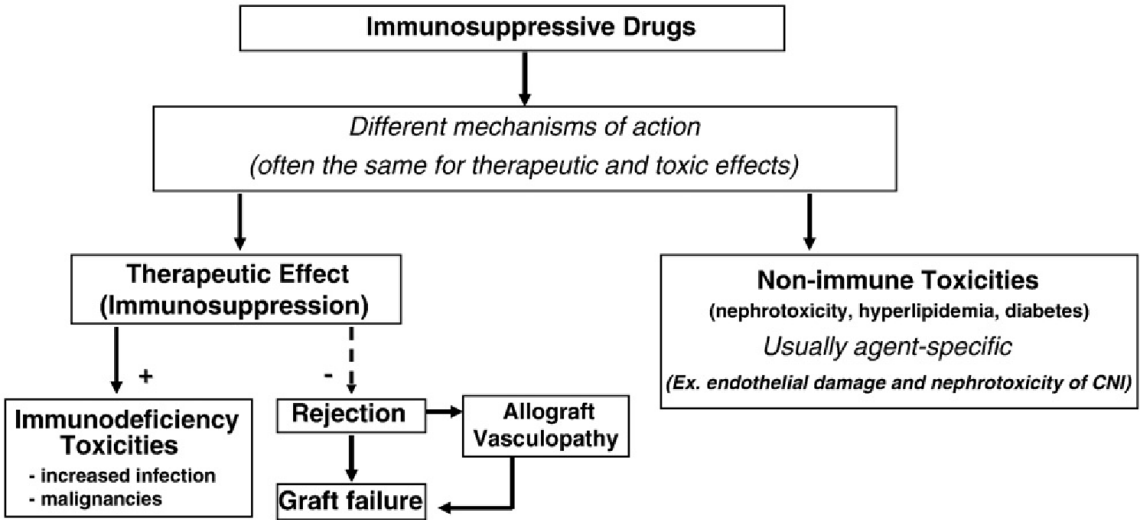

Figure 1. Effects of immunosuppressive drugs affecting graft and patient survival after Heart Transplantation (5).

Immunosuppression regimens are generally defined as induction, maintenance, and rejection regimens. Induction therapy is an intense perioperative immunosuppressive therapy because the

antidonor responses are typically most vigorous shortly after the transplantation, when stimuli such as donor brain death, ischemia/reperfusion and surgical trauma increase donor antigen expression, thus augmenting the recipient's immune response. The benefits of induction therapy are a marked reduction in rejection in the early postoperative period, when graft dysfunction and renal dysfunction are problematic (5).

Maintenance therapy generally consists of combination therapy with an antimetabolite, a calcineurin inhibitor, and steroids. Combination therapy targets several steps in T-cell activation, allowing lower doses of each individual drug. Specific maintenance regimens vary at individual transplantation centres and are based on age, presensitization and previous rejection, because each of these factors determines a patient's risk for rejection. Early maintenance therapy generally consists of a steroid, a calcineurin inhibitor with either cyclosporine or tacrolimus, and mycophenolate mofetil. Therapy is gradually decreased over time, and the late maintenance consists in a corticosteroid withdrawal, within 6 months after HTx, and cyclosporine serum concentration lowering, guided by serial endomyocardial biopsies coupled with clinical and laboratory findings (5, 6).

Finally, rejection (or rescue) therapy refers to immunosuppressive therapy given to reverse an episode of rejection. The intensity and type of rejection therapy depend on the severity and hemodynamic consequences of the rejection, whether it is thought to be T-cell mediated or humoral, as well as centre-specific protocols (7).

Even if immunosuppressive drugs have steadily evolved from the poorly efficacious and toxic agents used in transplantation's early days, and many advances have been made on the appropriate selection and careful monitoring of these drugs (blood levels, effects, and side effects) (Fig. 2), they are still cause of many adverse effects (Table 1).

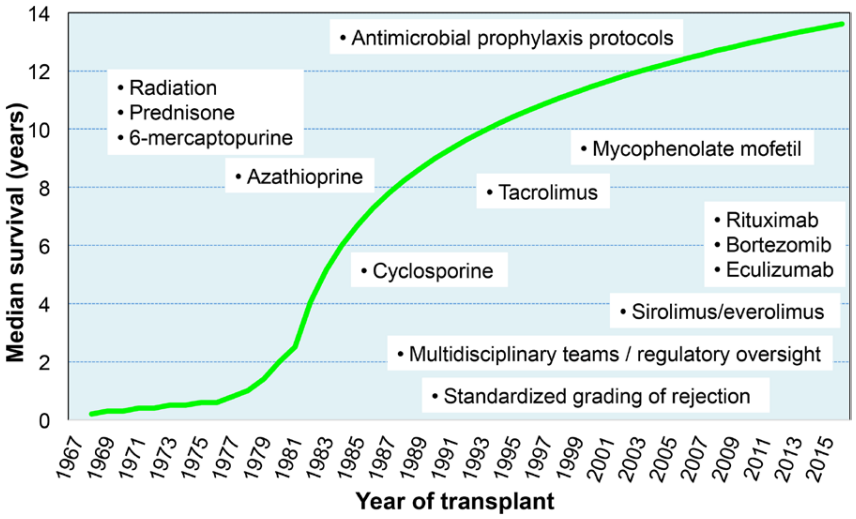

Figure 2. Median survival after heart transplantation and approximate time of introduction of key immunosuppressive agents and standardized clinical care approaches in heart transplantation based on data submitted to the ISHLT Registry (4).

1.2.1 Calcineurin Inhibitors

The calcineurin inhibitors lead to inhibition of interleukin-2 transcription, which ultimately interferes with T-cell proliferation. Moreover, these drugs have a direct effect on free and intramuscular calcium ion concentrations through the involvement of the calcium-calmodulin–dependent phosphatase mechanism. Increased calcium within the vascular smooth muscle leads to constriction and arterial hypertension, one of the most common side effects of calcineurin inhibitors, especially cyclosporine (8). Other metabolic side effects related to cyclosporine use are hyperlipidaemia and de novo diabetes mellitus at 1 year, which is present as many as 10% of patients, as long as a higher risk to develop osteoporosis (5). It is also thought that cyclosporine may have a role in increasing the endothelin receptors in the renal microvessels, which in turn produces vasoconstriction and renal-modulated hypertension. In addition, the marker Endothelin-1 seems to contribute to stimulation of proinflammatory cytokines, tissue damage, and fibrosis seen in patients after transplantation (8). For most of the patients, cyclosporine represents the first line calcineurin inhibitor and is taken twice daily.

The other calcineurin inhibitor, Tacrolimus, functions in a similar manner to cyclosporine and represents the second line of choice, when cardiologists face persistent recipient rejection episodes. Even if Tacrolimus is better tolerated and results in lower rates of hypertension and hyperlipidaemia, it increases the risk of diabetes mellitus. Rates of nephrotoxicity, neurotoxicity, infection, and malignancy are comparable between the two drugs (9).

1.2.2 Cell Cycle Inhibitors

Similar to calcineurin inhibitors, cell cycle inhibitors are generally taken life-long. Mycophenolate Mofetil (MMF) is used first line in heart recipients: when metabolised to mycophenolic acid, MMF inhibits purine nucleotide synthesis pathways required for T and B-cell proliferation, antibody formation and cell-mediated immune responses. As regards the side effects, MMF can cause especially leukopenia, but it is also associated to gastro-intestinal disturbances such as nausea, vomiting and diarrhoea. In addition, various studies demonstrate negative changes in lipid profile when MMF is included in immunosuppressive triple therapy (5, 10).

1.2.3 Glucocorticoids

Steroids, among the first immunosuppressive agents used in transplantation, have remained an important component of induction, maintenance, and rejection regimens. They are associated with the largest number of long-term adverse effects, first of all post-transplant weight gain. Glucocorticoids are known to disrupt fluid and electrolyte balance, which may in part explain patients' perception of weight gain, but they are also associated with increased appetite. Moreover, glucose dysregulation and insulin resistance can occur in a dose-dependent manner with both short and long-term administration, which may contribute to patient-reported food cravings (10). Other associated adverse effects are hypertension, hyperlipidaemia, osteopenia, emotional lability, cataracts, gastric ulcer. These drugs have also consistent cosmetic effects, that include hirsutism, acne, easy bruising, skin fragility, moon face, buffalo hump and truncal obesity (5).

	CYA	**TAC**	**Steroids**
Hypertension	4	3	2
Diabetes	1-2	2-3	3
Obesity			2
Hyperlipidemia	3	3	2
Osteoporosis	1-2	1-2	3
Renal Insufficiency	3	3	

Table 1. Most common adverse effects of the pricipal immunosuppressive drugs. CYA: Cyclosporine A; TAC: Tacrolimus. 1=Rare (<5%); 2=Common (5-15%); 3=Very common; 4=Most patients. (6)

1.3 Common complications after heart transplantation

Heart transplantation recipients are exposed to the risk of several potential complications that may impair their outcomes. The next paragraphs will provide an overview on the most common complications in the long-term period after cardiac transplant.

1.3.1 Acute Allograft Rejection

Although the improving of immunosuppressive regimen has decreased its incidence over the years, acute allograft rejection still accounts for about 5% of deaths occurring in the first 30 days after transplantation, and 11% of deaths within the third year. After this point, its incidence decreases to only about 2% of deaths (1). The pathogenesis of rejection is related to various pathways of allo-immune response of host immune system against graft antigens, mainly (but not only) represented by the human leukocyte antigen (HLA) specificity mismatches. Currently, two mechanisms are recognized leading to graft injury during acute rejection: cellular-mediated rejection and antibody-mediated rejection (11).

In cellular-mediated rejection, there is a prevalent activation of the T-cell compartment, leading to T cell-mediated cytotoxicity of myocardial tissue. This process has been defined and classified by descriptive pathological criteria defining the extent, the distribution, and the presence of myocyte damage associated with the detection of mononuclear cells infiltrating the myocardium in endomyocardial biopsies (12).

Antibody-mediated rejection (AMR) is characterized by circulating antibodies directed against antigens expressed by endothelial cells. The injury may depend upon complement activation but also upon complement-independent inflammatory pathways activated within the endothelial cells or mediated by natural killer cells. This kind of rejection has been recognized and tentatively classified only recently, and the pathological classification, although helpful in defining the diagnosis, is not sufficient to trigger treatment, which is itself not standardized (11).

1.3.2 Infection

Infections are the most relevant complications after heart transplantation and are among the most frequent cause of death along the entire post-transplant follow-up (11). Immunosuppression therapy places the transplant recipients at higher risk of infection, and, although the use of selective antimicrobial prophylaxis and advances in immunosuppression have reduced the risk, infections remain an important cause of post-transplantation mortality. Up to 13.4% of deaths in the first month

after transplantation are attributed to non-Cytomegalovirus (CMV) infections, with bacterial septicaemia the predominant infection. During the first year after transplantation, mortality from infections remains high (31.3%), but subsequently declines to approximately 10-13% per year (3).
In the long-term, pneumonia (bacterial), urinary tract infections (often bacterial), cytomegalovirus (CMV) infections, herpes virus or varicella-zoster infections, and mycosis may frequently occur (11). Viral infections, especially CMV, are a major cause of morbidity and mortality, with an incidence of CMV affecting up to 80% of heart recipients, depending on donor-recipient serostatus and intensity of the immunosuppressive regimen. CMV is a member of the β-herpesviridae family that includes human herpesvirus-6 (HHV-6) and HHV-7. Following transplantation, immunosuppression and systemic inflammation may promote the reactivation of the latent virus (either of donor or recipient origin), misbalancing the host–virus equilibrium. This imbalance may lead to both direct CMV cytotoxic effects and the so-called indirect CMV effects, which are believed to be a consequence of the complex immunomodulatory and pro-inflammatory events triggered by the virus: CAV is a typical example of indirect effect of CMV infection (11).

1.3.3 Chronic kidney disease

The second most common complication after heart transplantation is represented by renal failure. Hamour et al. reported that chronic kidney disease (defined by an estimated glomerular filtration rate (eGFR) of <60), occurred at a rate of 44% within one year of transplantation, and the incidence of serious renal impairment increased progressively with time (13). In the ISHLT registry is reported a 27% cumulative probability of developing severe renal dysfunction (creatinine >2.5 mg/dl, dialysis or renal transplant) by 5 years, 34% by 7 years and 42% by 10 years (14).
Impairment of renal function is influenced by aging and cardiovascular risk factors, such as diabetes and hypertension, but especially by immunosuppressive drugs, first of all cyclosporine (15). The use of calcineurin inhibitors is associated with a dose-dependent increase in blood urea nitrogen, serum creatinine, hyperkalaemia, metabolic acidosis, and chronic interstitial nephritis with irreversible renal toxicity. Reductions in the glomerular filtration rate are a direct result of decreased renal blood flow caused by these drugs. Although the mechanisms for renal injury from the use of CIs are not well-understood, histologically proven evidence of renal–vascular damage suggests direct nephrotoxic effects, leading to the loss of renal function and resultant hypertension (8).

1.3.4 Cardiac allograft vasculopathy

Cardiac allograft vasculopathy (CAV) is a chronic rejection of the transplanted heart and currently represents the main cause of graft failure and death after the first year of heart transplantation. Despite improvements in immunosuppressive drugs, the incidence of CAV has decreased only marginally, affecting up to 50% of recipients within 10 years of transplantation (4).

This disease is a rapidly progressive form of atherosclerosis characterized in its early stages by intimal proliferation and in its later stages by luminal stenosis of epicardial branches, occlusion of smaller arteries and myocardial infarction. It differs from atherosclerosis for a different pathophysiological process and the detailed pathogenesis is actually unknown, involving both immunologic and non-immunologic mechanisms (16). However, the classic cardiovascular risk factors, such as obesity, diabetes, dyslipidaemia, and hypertension, still have an important role in increasing CAV risk. Once CAV develops, current treatments are often ineffective, so prevention is important. The statins pravastatin and simvastatin started early after transplantation decrease the incidence of CAV. Other options are Vitamins C and E, which may also slow the progression of CAV. Aspirin is typically prescribed daily because of its established benefits in native coronary artery disease. Once CAV is detected, the introduction of a proliferation signal inhibitor such as sirolimus or everolimus can slow disease progression. Clinically significant CAV can be palliated with percutaneous coronary interventions for focal disease, but restenosis rates are high. Re-transplantation is often the only viable option but raises questions about equitable organ allocation (4).

1.3.5 Malignancy

Malignancies are a major threat to the long-term survival after heart transplantation. The ISHLT registry reports that the cumulative prevalence of malignancy at 1 year is 2.8%, while at 10 years reaches 28%, increasing steadily over the years from transplant (1). Immunosuppression significantly increments malignancy risk by globally reducing the efficiency of immune system in controlling for the onset of malignant cell clones, but also by direct carcinogenic mechanisms. Neoplasms virus-related are typical for these patients (e.g. non-Hodgkin's lymphomas and Epstein-Barr virus, Kaposi's sarcoma and HHV-6, skin cancer and papilloma virus). Indeed, epidemiological studies report that skin cancers, lymphomas, and prostate and lung cancer are more common in transplant recipients than in the general population. Tumours of skin may involve up to 20%, solid organ carcinomas (lung, gut, and prostate) up to 15%, and lymphomas up to 3 to 4% of the patients during the first 10 years post-transplant (11).

1.3.6 Metabolic alterations

Among the non-immune toxicity exerted by immunosuppressive treatment, the most relevant are represented by the metabolic alterations.

Overweight/Obesity

Post-transplant weight gain is the most common metabolic alteration, with an approximately 10 kg gain, on average, in the first year after cardiac transplant, which appears to be greater than the weight gain reported after renal and liver transplantation (17). Moreover, this weight gain tends to be progressive and is associated with the development of other metabolic complications.

Although many studies reported a severe impact by the glucocorticoid dose used (18), and considering all the above-mentioned side effects of these drugs, this association still appears to be controversial. Williams et al, for example, analysed the predictors of weight gain post-cardiac transplant, including age, gender and prednisone dose. Of all the factors, it was found only age to be a predictor of weight gain, with younger patients (age <48 years) gaining significantly more weight. Prednisone dose, instead, was not predictive of the amount of weight gain (17). As a matter of fact, though, the cause of weight gain is likely multifactorial, with immunosuppressive treatment playing a role, and this underlines the necessity to act on the other factors involved.

It is, indeed, reported that substantial weight gain after cardiac transplant increases the risk of secondary diseases, such as hypertension, diabetes and dyslipidaemia, and the association between body mass and patient morbidity and mortality has been noted both pre and post-transplant (10, 19, 20).

Hypertension

Data from the ISHLT show that hypertension is present in 50–90% of transplant patients and is associated with increased cardiovascular morbidity and mortality (1). One consequence of hypertension is increased arterial stiffness, which leads to cardiovascular events, CAV and increased mortality in transplantation patients (21). Hypertension as part of pre-transplant metabolic syndrome or its development within the first 3 months post-transplant, is reported to be associated with a greater risk of mortality and long-term renal dysfunction (8).

The development is multi-factorial but, once again, bears a distinct association with the use of immunosuppressive therapy: it is reported that the cumulative prevalence of hypertension with cyclosporine therapy is 52% at 1 year and 84% at 10 years after cardiac transplantation (22). It has been also shown that patients receiving cyclosporine develop new-onset hypertension requiring pharmacological treatment in 82% of cases compared with 64% of those treated with tacrolimus (8).

Hypertension, in cyclosporine-treated cardiac transplant recipients, is associated with reductions in cardiac output, increased vasoconstrictor sensitivity, decreased prostaglandin levels, and increased thromboxane A2 synthesis. Among the other immunosuppressive drugs, also glucocorticoids are reported to have a role in the development of hypertension, being the incidence of steroid-related hypertension around 15%. Several investigations suggest that glucocorticoids act on adrenergic receptors to potentiate the vascular actions of catecholamines and thus producing vasoconstriction (8). However, pre-existing hypertension significantly influences post-transplant progression, whilst donor family history may also contribute a developmental role (10).

Post-transplantation hypertension is frequently difficult to control and often requires a combination of several antihypertensive agents. Hypertensive heart recipients are sensitive to dietary sodium and restriction may offer a nonpharmacological role in blood pressure management. Moreover, postoperative heart recipients administered n-3 fatty acids at a dose of 4g per day, benefited from a prophylactic antihypertensive effect (10).

Dyslipidaemia

Dyslipidaemia is another common occurrence following heart transplantation, accounting for 60% to 80% of heart transplant recipients. Its pathogenesis involves elevated serum cholesterol; typically, elevated low-density lipoprotein (LDL) and triglyceride fractions coinciding with decreased high-density lipoprotein (HDL) (10). The development and progression of lipid abnormalities pose serious risk for future cardiac events. Hyperlipidaemia is thought to play a role in the development of CAV, cerebrovascular disease, and peripheral vascular disease.

Post-transplant hyperlipidaemia is a multifactorial phenomenon and has been attributed to genetic predisposition, diet, and diabetes, in addition to the use of immunosuppressive agents including cyclosporine and corticosteroids. The concentration of serum lipids after heart transplant are also influenced by many other nutritional, metabolic, and pharmacological factors, for example, the relative contribution of other medications, including diuretic and β-blocking agents (23).

The statins are as effective in reducing LDL-cholesterol in heart transplant recipients as in the nontransplant population. The benefits of statins in heart transplant recipients have been suggested to be even greater than in the general population and may be due to both cholesterol lowering and immune modulating effects (15).

Diabetes

The ISHLT Registry data showed that the incidence of post-transplant diabetes is 20% and 34% at 1 year and 5 years after heart transplantation, respectively (1). The primary risk factors influencing the

incidence of diabetes after transplantation are the commonly recognized factors for developing diabetes: overweight, obesity, positive family history, low physical activity, age >45 years, peer or ethnic groups at higher risk of diabetes, history of gestational diabetes or birth of a child weighing >4 kg, arterial hypertension, dyslipidaemias, polycystic ovary syndrome, and secondary hyperparathyroidism (24). Another major predisposing factor is immunosuppressive therapy, including glucocorticoid and calcineurin inhibitors (19). In particular, Tacrolimus is associated with a higher incidence of post-transplantation diabetes than Cyclosporine, especially when used in higher doses (15, 25). Finally, other risk factors include CMV infection, hepatitis C, male sex of the recipient and donor, and immunological reactions of acute rejection (24). As regard obesity, an increased BMI before heart transplantation was reported by Zhao et al to be an independent risk factor for post-transplantation diabetes (19), while Zielinska et al suggested that increase in body mass following a heart transplant can be a significant factor influencing the occurrence of this metabolic alteration (24). Diabetes is associated with significantly increased risk of cardiovascular and neuropathic complications, and improper glycaemic control and management may severely impact survival (10). In the study by Zhao et al., indeed, post-transplantation diabetes increased the number of postoperative acute rejection episodes and the rate of infection. Moreover, the all-cause mortality rate was 2.65 times higher in patients with than without post-transplantation diabetes (19).

Osteoporosis

Osteoporosis resulting in vertebral fractures is a common and debilitating problem after heart transplantation. The cause is multifactorial, compounded by the nearly 50% pre-transplantation prevalence of osteopenia and osteoporosis in patients with advanced heart failure. Glucocorticoids are the major factor in additional bone loss after transplantation, with contributions from renal insufficiency and Calcineurin inhibitors. Two years after heart transplantation, as many as 28% of recipients have osteoporosis in the lumbar spine, with vertebral fractures reported in up to 30%. Most bone loss occurs in the first 6 to 12 months after transplantation when steroid doses are highest. Several, studies suggest that bisphosphonates can prevent bone loss and fractures after cardiac and liver transplantation. Recommendations for patients receiving >5 mg/d prednisone for 3 months include calcium (1500mg/d) and vitamin D (800 IU/d), regular weight-bearing exercise, and a bisphosphonate (15).

To sum up, a final point to underline is that heart recipients rarely encounter one of the afore mentioned metabolic factors isolated; rather, a clustering pattern exists that includes two or even more

of them. This pattern has been termed ‘metabolic syndrome’ and its development post-transplant may significantly impact long-term survival.

1.4 Metabolic syndrome in heart transplantation

The last decades changes in lifestyles and dietary habits have produced a dramatic increase in the prevalence of obesity and related risk factors, including diabetes, hypertension and metabolic syndrome (26). Metabolic syndrome is an association of cardiovascular risk factors, such as elevated glucose, hypertension, abdominal obesity, and dyslipidaemias, that cluster in the same subject. Being a multifactorial disease, the physiopathological process of its development is really complex, but insulin resistance and abdominal obesity are proved to play a key role (27).

Metabolic syndrome was defined by the Adult Treatment Panel III (ATP III) as the presence of three or more of the following conditions: abdominal obesity (waist circumference >102 cm in men and >88 cm in women), hypertriglyceridemia (>150 mg/dL, 1.69 mmol/L), low high-density lipoprotein levels (HDL; <40 mg/dL, 1.04 mmol/L in men and <50 mg/dL (1.29 mmol/L) in women), high blood pressure (>130/85 mm Hg), and high fasting glucose levels (>110 mg/dL, >6.1 mmol/L) (28).

The presence of metabolic syndrome has been associated with a twofold increase in the risk of development of cardiovascular disease, cardiovascular mortality, and nonfatal acute myocardial infarction and stroke, and a 1.5-fold increase in all-cause mortality (29). Moreover, this chronic inflammation state observed in metabolic syndrome is itself cause of other associated sever pathologies, such as cancer, neurological disorder and non-alcoholic steatohepatitis (Fig. 3) (30).

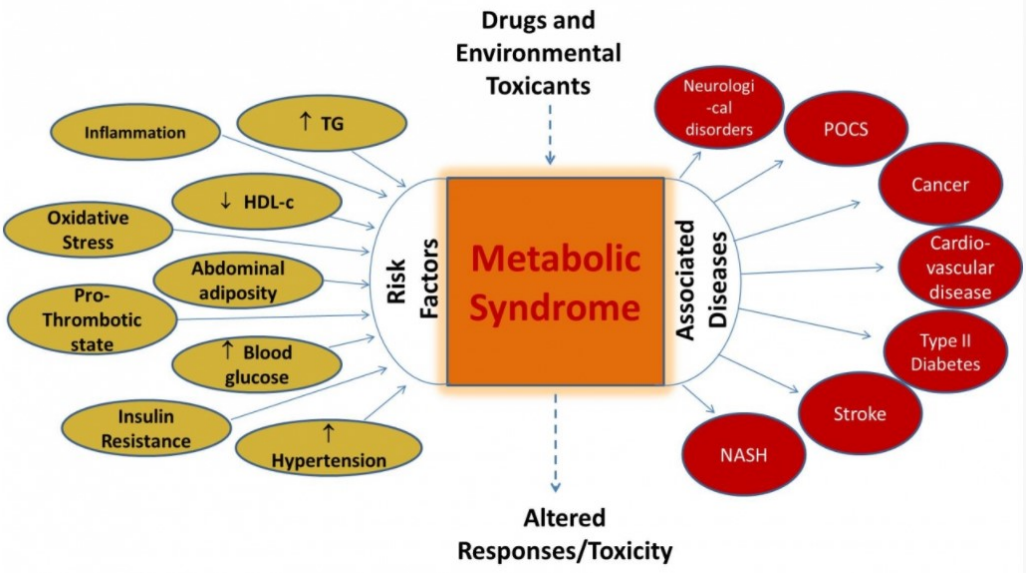

Figure 3. Metabolic syndrome with its associated risk factors and diseases at the intersection with drug toxicity (TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; PCOS, polycystic ovary syndrome; NASH, nonalcoholic steatohepatitis) (30).

1.4.1 The physiopathological processes of Metabolic syndrome

There are four main players involved in the initiation and progression of metabolic syndrome: (a) insulin resistance, (b) metabolic inflexibility and mitochondrial dysfunction, (c) adiposopathy, and (d) inflammation (31).

Insulin resistance

Insulin resistance is the consequence of a decrease in insulin responsiveness in key metabolic tissues, such as skeletal muscle, fat, and liver, evoking an abnormal response of cells of these tissues from hormone stimulation. Thus, insulin resistance exacerbates the deregulation of feedback mechanisms and exposes an organism to a pathological status. First of all, the impairment in the insulin-mediated inhibition of lipolysis, occurring in adipose tissue, promotes an increase in serum levels of free fatty acids (FFAs) (32, 33). Circulating FFAs, in turn, inhibit protein kinase in muscle cells, reducing their glucose uptake and, moreover, promote gluconeogenesis and lipogenesis in the liver (34). The systemic consequence of these features leads to a hyperinsulinemic state to maintain the glycaemic balance, but, if this reparation fails, insulin secretion decreases. One of the consequences is endothelial cells dysfunction. Physiologically, insulin-binding-mediated signalling results in the activation of two pathways, the PI3K–Akt pathway and the mitogen-activated protein kinase (MAPK) pathway (35). However, during insulin resistance, the balance between these two pathways is lost. Particularly, PI3K–Akt pathway is inhibited, resulting in a reduction in endothelial nitric oxide (NO) production in vascular cells. Insulin-induced NO, in physiological conditions, is responsible for the increase in blood flow, which is useful to enhance glucose uptake in skeletal muscle (36). On the other hand, the MAPK pathway is unaffected by insulin resistance, thus resulting in endothelin-1 secretion and VCAM and E-selectin expression, which contribute to leukocyte–endothelial interactions and mitogen stimulation for vascular smooth muscle cells. Overall, this insulin resistance-mediated unbalance may lead to vascular abnormalities predisposing to atherosclerosis (31). Insulin resistance is strictly associated with atherogenic dyslipidaemia due to the FFA spillover from the liver to the bloodstream. This leads to an increase in triglycerides synthesis along with an augment in the production of apolipoprotein B (apoB) containing very low-density lipoprotein (VLDL). Physiologically, insulin contributes to apoB degradation by acting on the PI3K-dependent pathway, thus impairing VLDL production and circulation. On the contrary, insulin resistance mediates the increase in triglyceride-rich VLDL, which leads to an increase in LDL and a concomitant decrease in HDL, thus influencing the development of atherosclerosis (31, 33).

Metabolic inflexibility and mitochondrial dysfunction

Another feature of metabolic syndrome is metabolic inflexibility. Metabolic flexibility is the physiological process by which an organism adapts fuel oxidation in response to changes in nutrient availability: during fasting, healthy subjects predominantly meet energetic demand by lipid oxidation and fatty acids (FA) uptake; after meals, under the insulin-stimulated effect, the organism shifts toward glucose, by activation of uptake, oxidative, and storage pathways along with the consequent suppression of lipid oxidation (37). In metabolic syndrome, an altered insulin-mediated substrate switching compromises this dynamic plasticity: during fasting, insulin resistance patients show a reduced ability of skeletal muscle cells to switch toward FA oxidation. As a matter of fact, insulin resistance is also associated with an impaired functionality of mitochondria, which have a primary role in FA oxidation. Abnormalities in mitochondria morphology, numbers, and functionality have been described in skeletal muscle cells of insulin resistance patients. Unhealthy mitochondria jeopardize FA β-oxidation, causing a lipid overload in adipocytes with a relative increase in stress signals and proinflammatory mediators (31, 38).

Adiposopathy

Adipose tissue has recently emerged as an active organ, showing endocrine and immune features and, thus, playing a role in the development of metabolic syndrome. In particular, white adipose tissue is the "endocrinal" component, especially the visceral adipose tissue, which secretes adipokines, biologically active molecules associated with metabolic regulation. Although visceral adipose tissue accounts for only 12–20% of total body fat, it is strictly associated with metabolic syndrome and cardiovascular disease. The adipokine Leptin, for example, controls energy homeostasis, and it has been known to have a proinflammatory activity by stimulating the Th1 pathway. On the other hand, Adiponectin is showing anti-inflammatory and antiatherogenic properties that contribute to a decrease in vascular reactivity. Researchers have shown that an increase in visceral adipose tissue correlates with an increase in leptin levels along with concomitant decrease in adiponectin levels, thus exposing organisms to cardiovascular risk (39, 40).

An increase in adipose tissue mass is also associated with an augmented production of angiotensin II (Ang II), a key player in the renin–angiotensin system, which is strictly regulate blood pressure and fluid balance. In addition, Ang II contributes to the production of reactive oxide species (ROS) and leads to the increase of other sources of oxidative stress, an important cause in the pathogenesis of metabolic syndrome, by triggering or exacerbating many biochemical processes: low-density lipoprotein (LDL) oxidation, expression of the redox sensitive NF-κB and platelet aggregation. Together, these processes contribute to signal cascades leading to dyslipidaemia, diabetes, and cardiovascular disease (41).

Adipocyte hypertrophy is another feature induced by a hypercaloric state and the increase in fat storage predisposes to intracellular hypoxia that, in turn, mediates the release of FFAs into the bloodstream. This induces fat deposition in non-adipose sites, such as liver, muscle, pancreas, kidney, and blood vessels, causing lipotoxicity. Lipotoxicity manifested at the muscular level induces insulin resistance. Overall, lipotoxicity contributes to the exacerbation of metabolic syndrome features (42, 43). The anatomic and functional alterations just described above are accountable for the induction of "adiposopathy," which in turn contributes to the worsening of metabolic syndrome and increase in cardiovascular risk factors (31).

Inflammation

Metabolic syndrome is characterized by a "low-grade" chronic state inflammation, commonly called "meta-inflammation." As previously described, obesity-induced oxidative stress and insulin resistance activate a signalling cascade having a proinflammatory effect. Visceral adipose tissue is also accountable for the release of many proinflammatory adipokines and cytokines, such as TNF-α, IL-6, IL-8, production of plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein (CRP) (44). It has been documented that IL-6 production by adipocytes increases with the rise of body fat and insulin resistance. IL-6, in turn, stimulates the production of CRP, the most commonly used marker to assess systemic inflammation. Experimental findings show a direct relationship between elevated levels of CRP and development of metabolic syndrome, diabetes, and cardiovascular disease (33, 45).

Finally, the ability of lipids to interact with cell surface receptors, such as Toll-like receptors, emerges as further evidence in triggering the signal cascade response to inflammatory process activation (31).

1.4.2 Prevalence of Metabolic syndrome

In the general population, the prevalence of metabolic syndrome varied from 18% to 39% depending on the diagnostic criteria used, demographic, and racial differences. Its prevalence in the heart failure population is higher than in the general adult population (20, 27), as long as among patients undergoing solid organ transplantation, where the prevalence varied from 28.6% to 63% (29). Particularly, this syndrome is highly prevalent in heart transplanted patients, both before and after the surgery. Martinez-Dolz and colleagues reported a prevalence of early metabolic syndrome (pre-heart transplantation or in the first 3 months post-transplant) of 41.9% (46).

As previously described, obesity, especially visceral one, is very common in these patients, and waist circumference, rather than BMI, is strongly linked to the syndrome (26). Moreover, metabolic

syndrome, but more specifically the insulin resistance, is associated with the presence and progression of CAV, renal dysfunction and long-term mortality (26, 29, 47, 48).

1.4.3 Metabolic syndrome and CAV

Systemic inflammation represents an important mechanistic pathway through which metabolic risk factors lead to vascular disease, and in heart transplanted patients this results in an increased risk to develop CAV. In a study by Biadi and colleagues it was shown that an impaired insulin sensitivity and increased systemic inflammation identify patients with a higher prevalence of angiographic CAV, independently from the time after transplant (49). In the study, the authors considered CRP as marker to summarize the level of systemic inflammation and the ratio between the serum concentration of triglycerides and high-density lipoproteins (TG/HDL) > 3.0 as an indirect and widely available marker of insulin resistance. On this basis, they found out that high CRP and TG/HDL of 3.0 or more independently predict an increased risk for major cardiac adverse events (MACE), suggesting an interaction effect between systemic inflammation and insulin resistance in worsening cardiovascular prognosis after heart transplantation (49). These data were successively confirmed also from other studies (50). The ratio TG/HDL appeared to be as an easy-to-use marker to identify heart recipients with CAV and with poor cardiovascular prognosis, as already mentioned above (26, 49, 50).
As a matter of fact, elevated CRP is not only associated with systemic inflammation but has also been reported to precede diabetes mellitus and to be an early marker for the development of the metabolic syndrome (49-52). Therefore, it appears clear that a chronic persistent systemic inflammation and the metabolic syndrome, together with renal dysfunction, are important independent factors in determining cardiovascular risk after heart transplantation.

1.4.4 The importance of a healthy diet

Given the huge number of daily drugs required to maintain a transplant patient, further therapeutic interventions to monitor metabolic disorders are probably not ideal. This is where a modification of patient's lifestyle, through a proper dietary intervention, together with a correct physical activity program, might offer a potential long-term benefit for this unique population.
As a matter of fact, Lee at al. reported that adherence to both physical activity and dietary guidelines is associated with a lower odds of metabolic syndrome, as long as a lower risk to develop metabolic syndrome prospectively, in 2379 Framingham Heart Study participants (mean age 47 years; 54.4% women) (53).

Several major institutions, including the World Health Organization(54), identified diet and nutrition as major avenues to address chronic diseases amongst the general population. Dietary modification is fundamental to prevent obesity, however, nutritional support following transplantation appears currently sub-optimal and dietary advices are often too general and provided without practical information. While recommendations for post-transplant diet and nutrition currently focus on the acute phase post-transplant in the effort to promote healing, help to prevent infection, support metabolic demands and replenish lost energy stores of recipients, no further advices are given to face metabolic disorders in the long-term period. In this case, in fact, the focus of dietary support should shift to the prevention of the metabolic syndrome, as explained above, while also managing chronic complications of graft rejection and immunotherapy.

Unfortunately, only a handful of short-term studies, usually in renal transplant recipients and encompassing only about 1 year in duration, have been published to date documenting an improvement in post-transplant metabolic syndrome through dietary recommendation. Moreover, only three studies involving cardiac transplanted patients have been published so far (Table 2).

For instance, Bellingheri and colleagues reported improvement in post-renal transplant BMI and metabolic syndrome during 1 year of a low-fat, hypocaloric diet, with consequent tapering of steroids (55). A low-fat diet is commonly defined with an approximate fat intake of 20-30% of total daily calories (10). In other European dietary intervention trials, using low fat and restricted calories during the first post-renal transplant year, body composition improved as demonstrated through decreased body fat, weight loss, lower serum cholesterol, improved fasting glucose, and increases in serum albumin (18, 56).

Other studies employing the American Heart Association (AHA) step one diet (57) with encouragement of physical activity have yielded similar reductions in lipid profile even in the setting of statin use. In AHA recommendations, the dietary plan is elaborated to fit an energy intake ≥25/kcal/kg/Ideal Body Weight/d, with 55% of carbohydrates, 15% of protein, and 30% of total fat (fatty acids <10% of calories and dietary cholesterol <300 mg/d). In their prospective study on heart transplant recipients, Guida et al. demonstrated a significant decrease in body weight, a reduction in BMI value and in triglyceride plasma level after only 1 year of AHA diet in compliant patients (58). Moreover, after the 48-month follow-up period planned, they showed a lower body weight, glucose, total cholesterol, and triglyceride plasma level. Plasma lipid level reductions were also noted in patients currently receiving statins to an additional 10% beyond the impact of the medication (58).

Etwistle and colleagues, instead, choose to compare two dietary regimens in their heart and lung transplanted patients: the low-fat diet and the Mediterranean diet (59). After 12 months of diet, the

Mediterranean diet group exhibited greater reductions in weight and BMI than those in the low-fat group, while the low-fat group showed a greater decline in heart rate and systolic and diastolic blood pressure. Insulin resistance, total cholesterol and triglycerides decreased with both diet interventions, while LDL declined more significantly in the Mediterranean diet group (60).

As regard in particular the Mediterranean diet, also a study from 1994 by Salen et al. noted numerous improvements in the dietary habits and metabolic markers of their cardiac transplanted population: the most marked change was the reduced intake of saturated fats with a conversely increased in the intake of monounsaturated fatty acids (MUFA). Regarding the biochemical variables, both total and low-density lipoprotein cholesterol were significantly reduced after diet modification (61).

Table 2. Summary of the prospective studies about dietary interventions in cardiac transplanted patients.

Ref. and year of publication	Dietary intervention	Number of patients	Timing of enrolment	FUP	Major Findings
Salen et al, 1994	French Med Diet	41	Not reported	18 months	Reduced intake of saturated fats. Significantly higher intake of MUFA. Significant decrease in total calories consumed. Both total and low-density lipoprotein cholesterol were significantly reduced.
Guida et al, 2009	AHA diet	42	20 within the first year, 22 after	4 years	Significant decrease in body weight, BMI, triglyceride plasma level, glucose, total cholesterol. Cholesterol level reduced by additional 10% in patients on statins.
Entwistle et al, 2020	Med diet and low-fat	10 and 10	1.43 (0.47–8.55) and 1.28 (0.45–5.95)	1 year	Med diet group: greater reductions in weight, BMI and LDL blood level. Low-fat group: greater decline in heart rate and systolic and diastolic blood pressure. Insulin resistance, total cholesterol and triglycerides decreased with both diet interventions.

1.5 Mediterranean diet

Among all dietary patterns, the Mediterranean diet has proved to be the most effective in reducing the cardiovascular risk factors. The evidence of its effectiveness lead back to the 50s, when Dr. Ancel Keys, an American physiologist and professor at the University of Minnesota, had an intuition about the relation about nutrition, metabolism and cardiovascular diseases in the South of Italy. Keys started the long-running Seven Countries Study, an authentic milestone in the large-scale epidemiologic research, which in the mid-1950s aimed to systematically explore the connection between cardiovascular disease, diet and nutritional habits in different geographical locations. The seven Countries involved were: Italy, Yugoslavia, Japan, Greece, Finland, Netherlands, USA. Keys observed that cardiovascular disease incidence was for instance higher in Finland, where dietary fat intake was high due to a large consumption of milk, meat and cheese. Conversely, the Japanese diet of the time was typically low in saturated fats and rich in fish consumption, and they demonstrated lower cardiovascular disease rates. Keys and colleagues also observed that the population of Crete (at that time) experienced the lowest rates of cardiovascular disease of all the participants. Incidentally, the Cretans ate a diet rich in fat, but its composition was primarily mono and polyunsaturated, comprising olive oil and fish (62). They noted that this particular diet pattern was typically consumed among Greece and Southern-Italy populations, traditional areas of olive cultivations, hence the name "Mediterranean diet". Adult life expectancy for population in these areas was among the highest in the world, and rates of coronary heart diseases, certain cancers and some other diet-related chronic diseases were among the lowest in the world in the early 1960s (63). Keys spent 40 years of his life living in South Italy, more precisely at Pioppi, Cilento, keeping on studying the local population nutritional habits and supporting the health benefit of the Mediterranean Diet.

The Mediterranean diet of the early 1960s was composed by an abundance of plant foods: fruits, vegetables, whole grains, potatoes, beans, nuts and seeds; minimally processed, seasonally fresh and locally grown foods; olive oil as the principal source of fat; dairy products (principally cheese and yogurt) consumed daily in low to moderate amounts; fish and poultry consumed in low to moderate amounts; red meats consumed in low amounts; and wine consumed in low to moderate amounts, normally with meals. It was, therefore, a diet low in saturated fat ($\leq$ 7-8% of energy), with total fat ranging from 25% to 35% (63). Fish products and the extra virgin olive oil (EVOO) provided, instead, essential fatty acids and oleic acid.

In more recent years, a Mediterranean diet pyramid was designed to better visualize the principal components of this traditional diet pattern and convey a general sense of relative proportions and

frequency of servings of food and food groups, modified in light of contemporary research (Figure 4).

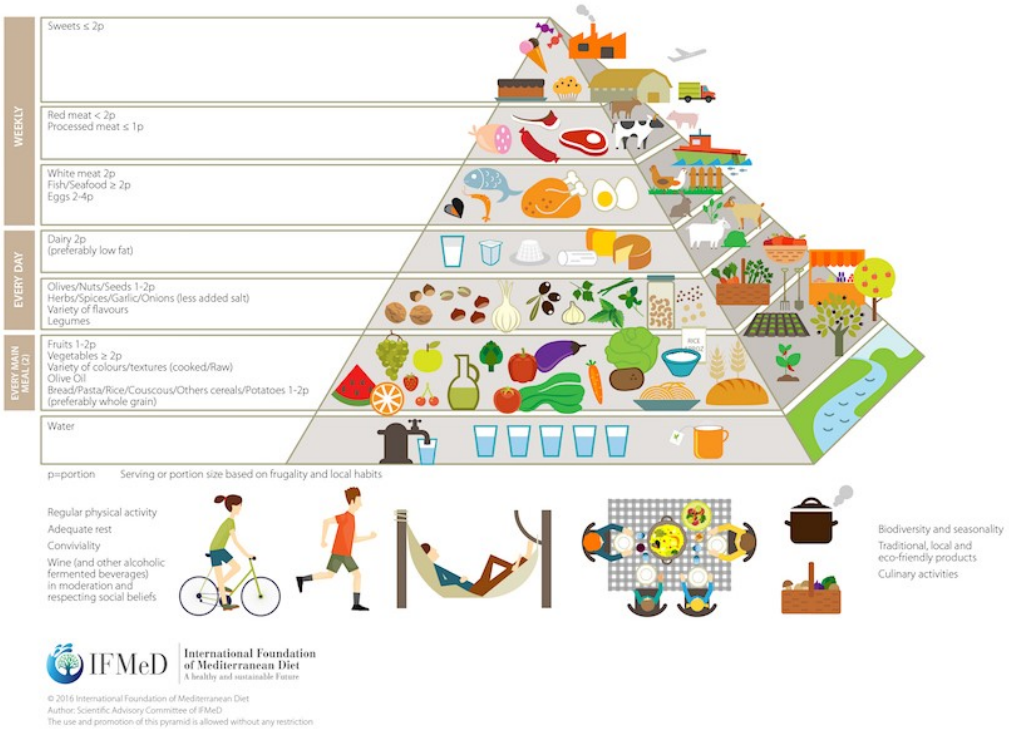

Figure 4. The most recent Mediterranean diet pyramid proposed by IFMED(64).

Speaking more in depth about the beneficial effect of Mediterranean diet, a huge number of studies have focused, all over the world, in evaluating any association between this diet pattern and various diseases. A recent meta-analysis of prospective cohort studies has demonstrated that greater adherence to a Mediterranean diet is associated with a 10% reduction in cardiovascular incidence or mortality and an 8% reduction in all-cause mortality in the general population (65). One of the main causes for the beneficial effect being discussed is the anti-inflammatory impact of Mediterranean diet leading to a reduction of inflammatory markers, in particular high-sensitive CRP (hsCRP) and IL-17a (66, 67).

In the 2000s, a Spanish multi-centre, randomized trial of people at high risk for cardiovascular disease, called PREDIMED (PREvenciòn con DIeta MEDiterrànea) study, showed that a Mediterranean diet reversed Metabolic syndrome more than a low-fat diet. The study involved 7447 people divided into three subgroups: a group composed by participants with Mediterranean diet supplemented with extra-virgin olive oil (EVOO), a group with a Mediterranean diet supplemented with nuts and a last group with low-fat diet (control group). The group with EVOO supplementation, after a median follow-up of 4.8 years, had reduced by 30% the rate of cardiovascular events, whereas the group with nuts had reduced by 28%, compared with the control group. Moreover, the

Mediterranean diet, either supplemented with EVOO or nuts, was not associated with the onset of Metabolic syndrome but only with its regression (68).

An interesting study by Esposito et al. has investigated the effect of a Mediterranean-Style Diet on endothelial dysfunction and markers of vascular inflammation in 180 patients affected by metabolic syndrome. Through a randomized, single-blind trial, the group who followed the Mediterranean-Style Diet had significant decreases in body weight, BMI, waist circumference, HOMA score for insulin resistance, blood pressure, levels of glucose, insulin, total cholesterol and triglycerides and a significant increase in levels of HDL-cholesterol, after two years of follow-up. Moreover, also serum concentrations of IL-6, IL-7, IL-18, and hs-CRP were significantly reduced in patients in the intervention group compared with those in the control group (69). Following this path, Kastorini et al. performed a meta-analysis evaluating 50 observational studies and clinical trails and confirming that the Mediterranean diet exerted favourable effects on the components of the metabolic syndrome in the adult population, reducing waist circumference, triglycerides, systolic blood pressure, diastolic blood pressure, glucose, and insulin resistance measured by homeostatic model assessment, and increases HDL levels (70).

An even bigger and more recent systemic review and meta-analysis by Papadaki and colleagues, including 2654 reports and 84 articles reporting 57 trials (n=36,983), showed that the Mediterranean diet resulted in greater beneficial changes in body weight, BMI, waist circumference, systolic and diastolic blood pressure, glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR) index, total-, LDL- and HDL-cholesterol, triglycerides, alanine transaminase, hepatic fat mass, CRP, IL-6, TNF-α, and flow-mediated dilatation, as long as lower risk of cardiovascular disease incidence and stroke in the general adult population (71). All these findings are confirmed by a number of other studies (72-74).

1.5.1 Mediterranean diet mechanism

The specific mechanism by which a Mediterranean-style diet can reduce the low-grade inflammatory state associated with the metabolic syndrome is complex. An important point to consider is the Mediterranean Diet works through the synergy of its constituent parts. The nutrients that are found in abundance in the Mediterranean diet have anti-cancer, anti-inflammatory and anti-obesity properties and contribute together to the maintenance of health status. However, many individual foods and nutrients that constitute this type of eating pattern do offer quantifiable health benefits (10).

Antioxidants-rich foods

The significance of oxidative stress in the transplant setting is extensive, as a consequence of both ischemia-reperfusion injuries and the metabolic alterations, as previously described. While a typical Western diet, rich in saturated fatty acids and red meat, can only worsened the oxidative stress already present in these patients, Mediterranean diet includes numerous foods with antioxidant properties. First of all, both fruits and vegetables are rich in Vitamins, and in particular Vitamin A, C and E, which have been proven to exert antioxidant affects (75).

However, the main class of nutrients with a widely studied and recognized antioxidant activity are the polyphenols (Figure 5). Phenolic compounds, also referred as polyphenols, are a heterogeneous group of molecules representing the most abundant secondary metabolites from plants used in dietary patterns, especially the Mediterranean diet. More than 8,000 different polyphenols have been described so far, each one showing property and bioavailability differences (31, 76, 77). Many studies have demonstrated that the anti-inflammatory effect observed following EVOO consumption seem to be attributed to its polyphenolic content (78). Clinical studies have disclosed the valuable results of olive oil consumption on endothelial function and inflammation markers with the antioxidant and anti-inflammatory properties of its polyphenols (79).

The polyphenols in red wine have antioxidant activity and cytoprotective action and have been proven to induce a change in the lipoprotein profile, in platelet aggregation, and in redox mechanisms. The wine and other derivatives of red grapes rich in resveratrol, a polyphenol stilbene, determine a vasodilatory effect through the endothelium-dependent up-regulation of nitric oxide (NO) production, and have a significant antioxidant activity. Resveratrol, found in grape skins, is a potent anti-inflammatory by inhibiting iNOS, COX-2 and NF-kB. Several studies confirmed that flavonoids, especially resveratrol, inhibit pre-adipocyte proliferation, adipogenic differentiation and de novo lipogenesis(42, 80, 81). In the PREDIMED population the moderate red wine consumption (≥ 1 drink/d) was associated with a lower prevalence of the metabolic syndrome in patients at a high cardiovascular risk (68).

Worth of mention is also a very typical Mediterranean diet food: tomato. Tomatoes are rich in lycopene, a carotene phytochemical which activates anti-oxidative enzymes and shows anti-inflammatory and insulin-sensing properties (42).

An unprocessed plant-based diet provides the richest source of exogenous antioxidants; coupled with the fact that transplant recipients experience continuous oxidative stress, an eating pattern based on these principles, like Mediterranean diet, may provide an opportunity to abrogate some of the problems associated with oxidative damage.

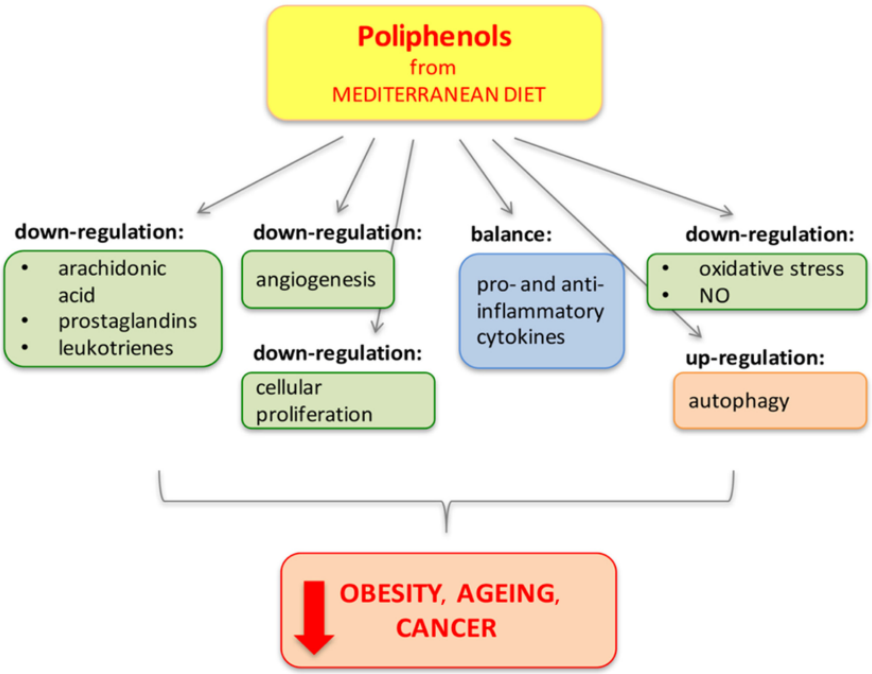

Figure 5. Polyphenols from Mediterranean Diet. Polyphenols protect and reduce inflammation by different pathways (through mechanisms of down-regulation, balance and up-regulation) preventing obesity, cancer and age-related diseases, in which inflammation has an important pathological role(42).

Fibres

Being a plant-based diet, Mediterranean diet is characterised of a high content of non-digestible carbohydrate, or fibres. Fibres are present in fruits, vegetables, whole grains and beans, all the pillars of this dietary pattern. This nutrient has many beneficial effects on health, including anti-inflammatory ones: increasing the fibre content (from 4.5 g to 16.8 g) of a high-carbohydrate meal is associated with significant reduction of circulating IL-18 levels in both healthy persons and patients with type 2 diabetes (82). Moreover, the fibres present during the meal delay the nutrients adsorption during the post-prandial phase, with a consequent delay of the adsorption of sugars and, thus, the glycaemic peak (83, 84). This action is really helpful in controlling the post-prandial glycaemia in patients with diabetes or insulin resistance (85, 86).

Another positive effect, especially for cardiac transplanted patients, is the gut microbiota modulation. As mentioned before, a common complication after heart transplant are infections, due to the immunosuppressive treatment. To counter this challenge patients' may be prescribed antibiotics, either acutely, or as prophylaxis. However, these drugs are not selective in destroying microorganisms and the commensal bacteria presented in our gastro-intestinal tract, the microbiota, are the inevitable side effect of an extensive use of antibiotics.

When in balance, microbiota is essential for human health and interacts symbiotically in a number of ways:

- Break down food to extract essential nutrients
- Produce vitamins that human genes are unable to produce
- Intimately involved in immune development and maintenance
- Manufacture anti-inflammatory compounds
- Ward off disease-triggering microbes

Antibiotic use significantly impacts intestinal homeostasis, and multiple exposure is linked to increased obesity, diabetes risk, and insulin sensitivity in patients with metabolic syndrome(10). Unfortunately, owing to continuous bacterial risk, antibiotic use in transplantation is a frequent necessity. Research demonstrates that the fibres have an influence in the structure of gut microbiota communities. These 'prebiotic' fibres act like a beneficial food for gut microbiota, restoring their environment and functions.

Omega-3

The Mediterranean diet is rich in Omega-3 polyunsaturated fatty acids (PUFAs), contained primarily in fish and seafood. Eicosapentaenoic acids (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) are the most important PUFA associated to cardio-protective effects, which can be explained by their modulate K, Na, and Ca channels activities in myocardial cells, regulating myocyte electrical excitability and contractility (42). Omega-3 have been proven to exert also favourable effects on body weight reduction and metabolic profile improvement. By a mechanistic point of view, omega-3 PUFAs induce the upregulation of transcriptional factors controlling adipogenesis, mediating a healthy expansion of adipose tissue upon feeding and contribute to a healthy metabolic phenotype (31). Moreover, omega-3 have also an anti-inflammatory activity, which is responsible for reduction of NF-κB transcriptional factor and its related pro-inflammatory cytokines IL-1, IL-6, and TNF-α, usually elevated in obese subjects (87). As a matter of fact, they play an important role in the prevention and treatment not only of cardiovascular diseases but also cancer, rheumatoid arthritis, psoriasis and cataract. Finally, omega-3 showed an activity in counteracting insulin resistance by improving glycogen synthesis. Studies in muscle of mouse fed with a high-fat diet evidenced the positive action of omega-3 in maintaining normal PI3K activity and GLUT4 expression levels, which lead to improve glucose uptake (88).

In conclusion, all these findings clearly suggest that the Mediterranean diet seems to be a safe and efficacy strategy for treatment of the metabolic syndrome in general population and, potentially, even more in all patients at higher risk of cardiovascular events, such as cardiac transplanted patients.

2 Research Hypotheses and Aims

Since metabolic syndrome represents a real burden in heart transplantation recipients, mainly due to the side effects of immunosuppressive therapy, acting on modifiable factors appears a valuable option to enhance the survival and quality of life of this patient population. Among these factors, a correct diet, especially the Mediterranean diet, has been proven to have a significant impact on preventing or limiting the progression of Metabolic Syndrome and all the associated metabolic risk factors. Unfortunately, a personalized dietary intervention in heart transplanted patients is not yet strongly rooted in their regular follow-up treatment.

Hypotheses:

1. Metabolic syndrome is highly present among cardiac transplanted patients followed at University Hospital of Udine.
2. Dietary habits of cardiac transplanted patients are not properly adherent to Mediterranean diet.
3. A structured nutritional education can be beneficial in terms of better dietary habits, anthropometric and body composition parameters, metabolic profile in this patient population.

Aims

1. Assess the presence of metabolic syndrome in heart transplanted patients of our centre
    a. Evaluate the impact on the long-term outcome of these patients in terms of morbidity and mortality
2. Analyse and describe the adherence to the Mediterranean diet after Heart transplantation in a sample of cardiac transplanted patients
3. Evaluate the impact of a structured personalized nutritional education in a group of cardiac transplanted patients, compared to a control group
    a. Evaluate change in adherence to the Mediterranean diet in the two groups
    b. Determine change in the clinical, anthropometric, body composition and blood parameters in the two groups
    c. Evaluate change in the dietary habits of cardiac transplanted patients who received the structured personalized nutritional education

3 Experimental part: Aim 1

Metabolic syndrome and heart transplantation: an underestimated risk factor?

The abstract of the study was presented as full oral presentation at the 44th National Congress of the Italian Society of Organ and Tissue Transplantation (SITO) in Naples (IT), at the 42nd Annual Meeting & Scientific Sessions of International Society of Heart and Lung Transplantation (ISHLT) in Boston (US), and at 53rd National Congress of ANMCO, Rimini (IT).

3.1 Aim

Assess the presence of metabolic syndrome in heart transplanted patients of our centre, and the impact on the long-term outcome of these patients in terms of morbidity and mortality.

3.2 Methods

3.2.1 Patient population and data collection

All cardiac transplanted (HTx) patients at University Hospital of Udine since 2007 were enrolled. Data were retrospectively collected from clinical informatic system and patient charts, considering 4 timepoints:

1. Baseline: before HTx surgery
2. At 1 year of follow-up after HTx
3. At 5 years of follow-up after HTx
4. At 10 years of follow-up after HTx

At baseline timepoint, demographic and clinical pre-HTx data were collected.
At the follow-up timepoints, long-term outcome and mortality, laboratory tests parameters, including a complete blood count, fasting blood glucose, lipid profile, renal function, echocardiogram exam parameters, drugs therapy, weight and blood pressure values were collected.

3.2.2 Follow-up and immunosuppression therapy

The postoperative and long-term follow-up protocol for HTx patients included endomyocardial biopsies made every week during the first month, every 15 days in Months 2 and 3, and monthly or bimonthly up to 12 months, and if required thereafter. Coronary angiography was performed at the first year and every 2 years afterwards or on clinical requirement. Clinical follow-up was conducted by a dedicated team including a cardiac surgeon, a cardiologist, a nurse and a psychologist every 15

days during the first 3 months, every month between Months 3 and 12, every 3 months between 1 and 3 years, every 4 months between 3 and 5 years and every 6 months after 6 years from transplantation. At each postoperative control, right and left ventricular function and morphology were evaluated by transthoracic 2D-Echo.

During clinical evaluation, adherence to immunosuppressive treatment was also verified, and therapy modified or titrated according to case-specific conditions. The first-line immunosuppression included cyclosporine, mycophenolate mofetil (MMF), and corticosteroids in all patients. All recipients received induction therapy with antithymocyte globulins, whenever possible. A standardized protocol for corticosteroid withdrawal, within 6 months after HTx, and cyclosporine serum concentration lowering was applied guided by serial endomyocardial biopsies coupled with clinical and laboratory findings.

3.2.3 Metabolic syndrome diagnosis

According to modified, revised NCEP-ATP III (Third Report of the National Cholesterol Education Program) criteria (28), diagnosis of metabolic syndrome (MetS) was made when the patient met at least three of the following criteria:

- Triglyceride levels ≥150 mg/dl or drug treatment for hypertriglyceridemia
- High-density lipoprotein (HDL)-C < 40 mg/dl in men and <50 mg/dl in women or drug treatment to raise HDL-C levels
- Diabetes mellitus (DM) and treatment for elevated glucose or fasting glucose levels ≥100 mg/dl
- Blood pressure ≥130/85 mm Hg or antihypertensive drug treatment
- Waist circumference >102 cm in men and >88 cm in women.
  This latter parameter was substituted with body mass index (BMI) > 30 as the cut-off point for obesity.

The diagnostic criteria used for MetS in this study have been used in various studies associating MetS with cardiovascular disease in both the general population and in HTx recipients (46, 47).

3.2.4 Definitions

Cardiac allograft vasculopathy (CAV) was diagnosed by angiography and defined according to the ISHLT classification (89). Infections were registered as any episodes requiring antibiotic treatment.

Malignancies included both haematological or involving solid organs. Rejection grade were calculated as described by Stewart et al (12).
Chronic kidney disease (CKD) was defined as stage 4 CKD according to an eGFR<30 mL/min/1.73mq, calculated through EPI-CKD equations.

3.2.5 Statistical analysis

Categorical variables were expressed as absolute frequency and percentage and quantitative variables as mean ± standard or median (interquartile range) according to data distribution, after performing the Kolmogorov-Smirnov test for normality.
Comparisons of continuous variables were made with Student's t-test for independent samples or the Mann–Whitney U test if the variables did not fit a normal distribution. Differences between percentages were compared using Pearson's chi-squared test with Fisher's correction when the number of expected values was less than five.
Overall survival was estimated using the Kaplan–Meier method (log-rank test). Cox-regression model estimated factors independently associated with long-term mortality grade ≥ 2 CAV. A difference was considered statistically significant if $p < 0.05$. All statistics were performed using the Statistical Package for Social Sciences (SPSS) program (Chicago, IL, USA).

3.3 Results

Since 2007, 349 patients underwent HTx at our centre. Baseline recipients data before HTx are presented in Table 3. Mean age was 56±11 years and 81% were men. The primary indication for HTx was dilated cardiomyopathy (DCM) in 48% of patients, followed by ischemic cardiomyopathy (ICM) in 28%, and other diseases in 23%. Smoking was present in 39% of patients, with 18% active smoker and 21% formers. Thirteen percent had chronic obstructive pulmonary disease (COPD) and 32% had chronic renal failure at the time of operation.

During a median follow-up of 53 (16-112) months, late mortality was 30%. Most common complications were infection episodes in 32% of patients, acute rejection grade ≥2 in 24%, malignancies in 19%, Cytomegalovirus (CMV) infection in 17%, renal failure grade ≥4 in 15% and CAV grade ≥2 in 9% (Table 4).

Table 3. Baseline recipients' data

N. patients	**349**
Mean Age, years	56±11
Male/Female, n (%)	283 (81) / 66 (19)
Aetiology	
DCM, n (%)	168 (48)
ICM, n (%)	97 (28)
Other, n (%)	79 (23)
Re-HTx, n (%)	19 (5)
Previous CCH, n (%)	127 (36)
Smoking, n (%)	137 (39)
Active smoker, n (%)	62 (18)
Former smoker, n (%)	75 (21)
COPD, n (%)	13 (4)
Chronic Renal Failure, n (%)	112 (32)
Atrial fibrillation, n (%)	57 (16)
PM/ICD, n (%)	110 (32)
MCS, n (%)	72 (21)
Median LVEF, %	27 (20-35)
Mean sPAP, mmHg	43±1

CCH = cardiac surgery; COPD = chronic obstructive pulmonary disease; DCM = dilated cardiomyopathy; ICD = implantable cardioverter-defibrillator; ICM = ischemic cardiomyopathy; HTx = heart transplantation; LVEF = Left Ventricular Ejection Fraction; MCS = Mechanical Circulatory Support; PM = pacemaker; sPAP = systolic pulmonary artery pressure

Table 4. Long-term outcome

N. patients	**332**
Late mortality, n (%)	100 (30%)
Median follow-up, months	53 (16-112)
Acute rejection grade ≥ 2, n (%)	80 (24%)
Infection, n (%)	106 (32%)
CMV infection, n (%)	55 (17%)
Malignancies, n (%)	63 (19%)
CAV grade ≥ 2, n (%)	30 (9%)
Renal failure grade ≥ 4, n (%)	48 (15%)

CAV = cardiac allograft vasculopathy; CMV = Citomegalovirus

Metabolic syndrome

As regard the prevalence of MetS, 35% of patients had already the syndrome before HTx. During the follow-up, this percentage steadily grew, with 47% of patients within the first year after HTx and more than a half of them (52%) after 5 years of follow-up. The distribution of the incidence of MetS at 1 year after HTx remained stable during the study period, as observed in Figure 6.

Focusing on the singular criteria, half of the patients (50%) had hypertriglyceridemia before HTx, while the number had a dramatic surge during the follow-up, with 92% of patients at 1 year of follow-up, 89% at 5 years and 93% at 10 years. Another criterion, which has risen sharply was hypertension (HTN): 34% of patients had HTN prior to HTx, but the percentage reached 86% at 1 year of follow-up, 90% at 5 years and 91% at 10 years. Even if less alarmingly, also obesity increased during the follow-up: 12% of patients had a BMI > 30 before HTx, while within the first year after HTx the patients were the 19%, at 5 years 25% and at 10 years 20%. DM and glucose blood level appeared, instead, to be halved: while 61% of patients had DM or fasting hyperglycaemia pre-HTx, at 1, 5 and 10 years of follow-up the frequencies were respectively 35%, 43% and 38%. Finally, also HDL blood level, presented in 34% of patients before HTx, resulted decreased during the follow-up, with 18% of patients at 1 year, 20% at 5 years and 16% at 10 years (Table 5).

Table 5. Prevalence of Metabolic syndrome before and after Heart Transplantation

Prevalence of MetS	Pre-HTx	1-year f-up	5-year f-up	10-year F-up
N. of patients	349	280	166	80
TGL ≥150 mg/dl or hypertriglyceridemia drugs, n (%)	173 (50)	257 (92)	147 (89)	74 (93)
HDL <40 mg/dL in men or <50 mg/dL in women, n (%)	120 (34)	49 (18)	33 (20)	13 (16)
DM or gluc ≥100 mg/dL	211 (61)	97 (35)	72 (43)	30 (38)
Blood pressure ≥ 130/85 mmHg or HTN drugs, n (%)	120 (34)	242 (86)	149 (90)	73 (91)
BMI > 30, n (%)	43 (12)	53 (19)	41 (25)	16 (20)
MetS, n (%)	123 (35)	131 (47)	86 (52)	37 (46)

BMI = body mass index; DM = diabetes mellitus; HDL = high density lipoprotein cholesterol; HTN = hypertension; MetS = metabolic syndrome. TGL = triglyceride.

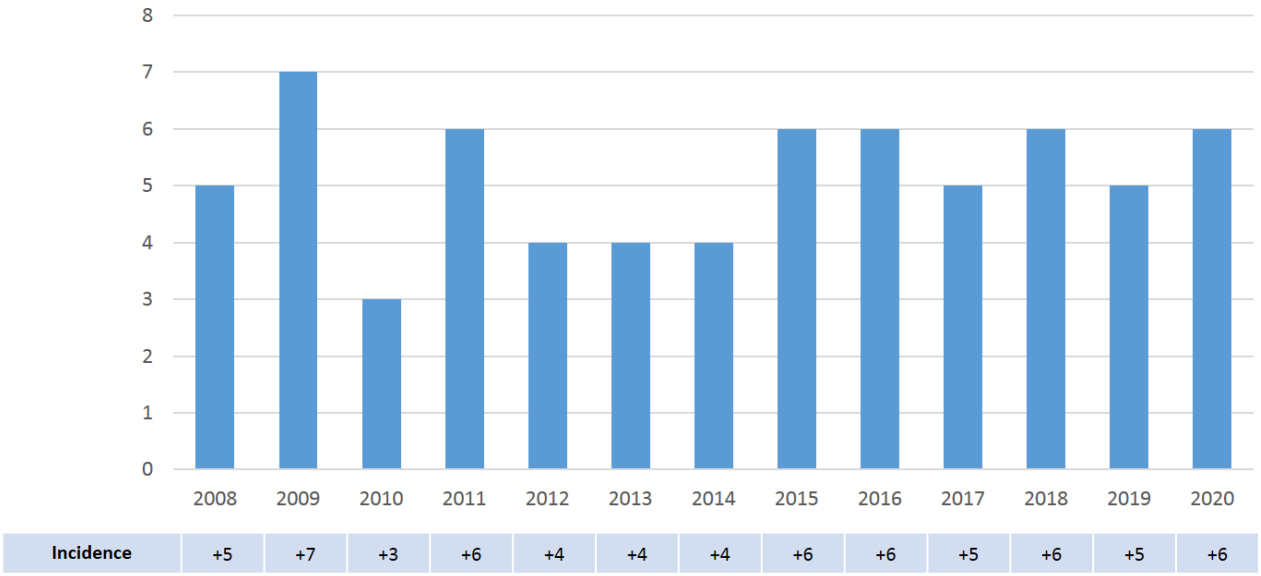

Figure 6. Distribution of the incidence of MetS at 1 year after HTx during the study period.

Mortality and morbidity predictors

Assessing more in depth the impact of MetS on outcome of cardiac transplanted patients, the overall survival in patients with MetS before HTx appeared significantly worst, resulting of 81±4% vs 90±2%, 65±5% vs 78±3% and 44±6% vs 66±4% ($p<0.01$) at 1, 5 and 10 years of follow up in patients with and without pre-HTx MetS, respectively (Figure 7). Similar results were found also in patients with MetS within the first year of follow-up, with a survival of 78±4% vs 89±3% and 57±6% vs 75±5% ($p<0.01$) at 5 and 10 years of follow up in patients with and without MetS at 1-year follow-up, respectively (Figure 8).

At the univariate analysis, risk factors for mortality were recipient age (HR 1.07, 1.04-1.09, $p<0.01$), pre-HTx MetS (HR 1.86, 1.29-2.69, $p<0.01$), pre-HTx HTN (HR 2.46, 1.70-3.55, $p<0.01$), pre-HTx hypertriglyceridemia (HR 1.50, 1.04-2.18, $p=0.03$), chronic renal failure (HR 2.95, 2.03-4.27, $p<0.01$), MetS and DM at 1-year follow-up (HR 2.00, 1.25-3.19, $p<0.01$; HR 2.02, 1.27-3.23, $p<0.01$, respectively). The last two resulted also risk factors for CAV (HR 1.86, 1.16-2.99, $p=0.01$; HR 1.67, 1.03-2.69, $p=0.04$, respectively). As a matter of fact, MetS at 1-year follow-up determined a significant higher risk to develop CAV at 5- and 10-year follow-up (25±4% vs 14±3% and 44±6% vs 25±4%, $p<0.01$) (Figure 9).

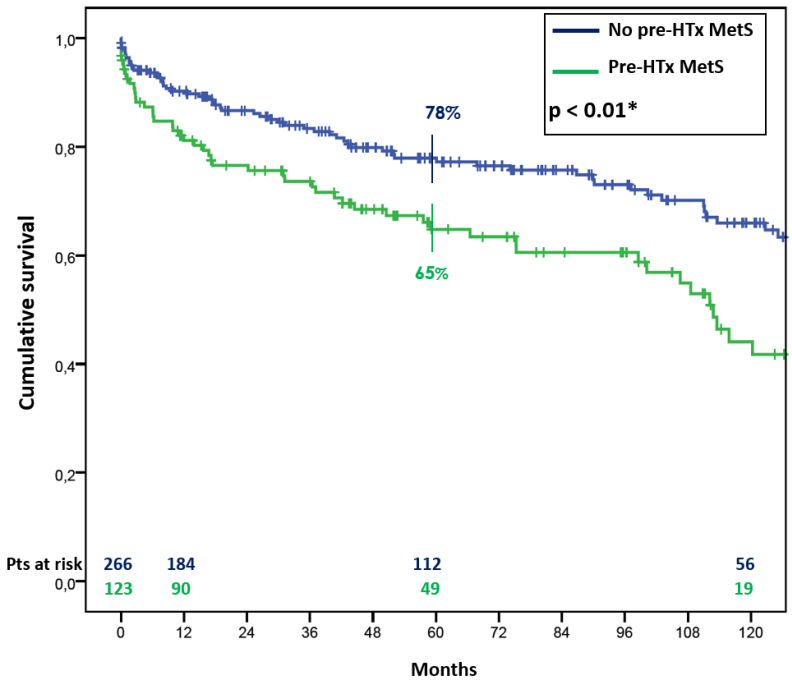

Figure 7. Cumulative survival in cardiac transplanted patients with or without MetS before HTx.

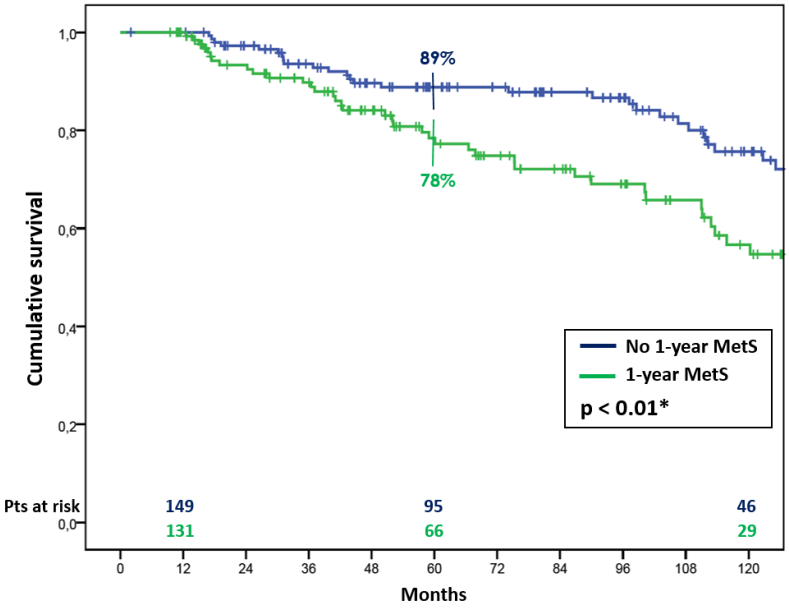

Figure 8. Cumulative survival in cardiac transplanted patients with or without MetS at 1 year after HTx.

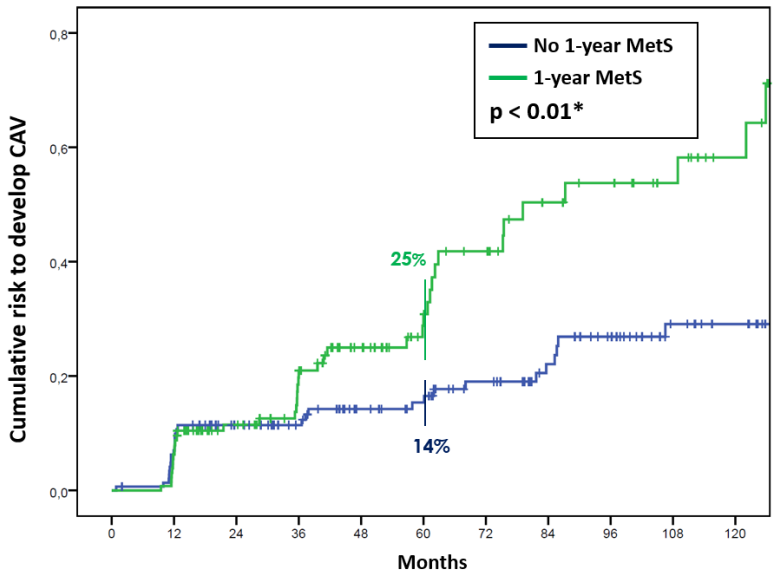

Figure 9. Cumulative risk to develop CAV in cardiac transplanted patients with or without MetS 1 year after HTx.

3.3.1 Discussion

The main findings of this study were 1) MetS was highly prevalent in HTx patients, with hypertriglyceridemia and hypertension being the most common increased metabolic factors; b) both MetS before and at 1 year after HTx determined a significative worst survival, resulting also risk factors for mortality at the univariate analysis; c) MetS at 1 year after HTx determined a significant higher risk to develop CAV in this patient population.

MetS is a burden that strongly impact long-term outcome of cardiac transplanted patients, and its prevalence was very relevant in HTx patients' population of this study, both prior and after HTx. In their TRACA study, Cordero et al. reported a 43% prevalence of MetS in HTx patients after 8±6 years from transplant (26). A similar percentage was also found by Martinez-Dolz and colleagues (46). However, the prevalence reported in our study was even higher, with more than half (52%) of the patients being affected by MetS at 5 years of follow-up. Moreover, the prevalence seemed to increase over the follow-up period, suggesting a possible association with greater exposure time to immunosuppressive medication.

Analysing the course over the follow-up of each of the parameters involved in the definition of MetS, it was observed a dramatic surge in blood pressure levels and triglycerides level, as long as an increase in BMI. These findings are probably directly related to immunosuppressive therapy and different

studies have shown a predisposition to the development of hypertension, dyslipidaemia and obesity in transplant population during the follow-up (1, 5, 23).

The presence of MetS has been associated with a twofold increase in the risk of development of cardiovascular disease, cardiovascular mortality, and nonfatal acute myocardial infarction and stroke, and a 1.5-fold increase in all-cause mortality (29). As a matter of fact, patients in this series who met early MetS criteria, both before and after 1 year of HTx, had significantly greater long-term mortality, accordingly to other studies reported in literature (46). Interestingly, also three of the MetS criteria analysed were found to be independent risk factors for mortality: HTN and hypertriglyceridemia prior to HTx and DM at 1-year follow-up. In particular, new onset DM after HTx has been reported to be associated to an increased risk of cardiovascular incidents resulting in death and other diseases. Other adverse effects included infection, rejection, and early graft loss (24).

In HTx patients, different metabolic abnormalities have been associated with the development of CAV or chronic rejection, which is one of the main causes of graft failure and death over the long-term follow-up after HTx (4). CAV is considered to be an accelerated form of atherosclerosis confined to the graft, caused by an endothelial dysfunction of multifactorial origin. Considering MetS is characterized by a chronic systemic inflammation that determines an endothelial dysfunction(31), it is reasonable to expect an impact of MetS on the development of CAV. Indeed, in this study it was found a significant association between MetS and DM at 1 year after HTx and CAV. A similar association was also found by Sanchez-Gomez and colleagues, where 67% of patients with MetS developed CAV, being the presence of MetS an independent predictor with an OR of 7.97 (29). At the univariate analysis, they found the MetS components hypertriglyceridemia, high BMI and low HDL-C levels to be associated with the development of CAV. In our study we found only DM to be an independent predictor, but considering that insulin resistance (IR) is a known cause of endothelial cells dysfunction and one of the main player involved in triggering MetS (31), a consequent correlation between all the other associated metabolic components appears clear. As a matter of fact, an association between IR and CAV was already been described in a study by Valantine et al (48). They showed that metabolic markers of IR are significantly correlated with coronary artery intimal thickening in the transplanted heart and that this metabolic abnormality significantly predicted the development of CAV and death during the subsequent 5 years of follow-up. Another interesting confirmation comes from a prospective, cross-sectional study by Raichlin et al, in which, evaluating blood samples from HTx patient on average nearly 5 years after transplant, markers of IR and

systemic inflammation independently identified patients at higher risk for subsequent angiographic CAV and cardiovascular events (50).

To sum up, MetS was confirmed to be highly prevalent also in HTx patients of our centre, causing a significant worst outcome in terms of survival and development of CAV. In particular, patients whit a diagnosed MetS before or within the first year of HTx should probably be followed closer, as they are more prone to develop cardiovascular events. Indeed, immunosuppressive therapy plays a primary role in the development and progression of MetS and the associated components. However, also not-appropriate dietary habits and physical inactivity may strongly impact on the metabolic status of these patients. Considering that, so far, the Mediterranean diet is the most recognised dietary approach to prevent cardiovascular event, the second step of this research will explore the adherence to the Mediterranean diet in a sample of HTx patients from our centre.

4 Experimental part: Aim 2

Adherence to the Mediterranean diet after Heart transplantation

4.1 Aim

Analyse and describe the adherence to the Mediterranean diet after Heart transplantation.

4.2 Methods

4.2.1 Patient population and data collection

Cardiac transplanted (HTx) patients who returned for the programmed follow-up visit at the HTx ambulatory at University Hospital of Udine since October 2020 were enrolled.

Clinical and demographic data were collected retrospectively from clinical informatic system and patient charts.

Dietary and physical activity habits were assessed using the questionnaire as described in the next paragraphs.

4.2.2 Dietary assessment

Adherence to the Mediterranean diet (MedDiet) was measured using the validate questionnaire proposed by Gnagnarella et al. (90). It is a short food frequency questionnaire (FFQ) with 14 questions to establish the frequency of consumption of 15 food items (Appendix A). A point is assigned for 10 of these items, which characterized key food groups commonly consumed in a traditional MedDiet, when consumed at an appropriate and established frequency. The answers were then summed to a total MedDiet score ranging from 0 to 9, with higher score indicating higher adherence. The results were finally ranked to estimate the adherence to the MedDiet as follows: score $\leq$ 5 point, weak adherence; score 6-9, good adherence (90, 91).

4.2.3 Physical Activity assessment

To assess physical activity the International Physical Activity Questionnaire (IPAQ) was used: seven items including intensity of activity (e.g., vigorous vs. moderate), the time spent walking in the last seven days, and the time spent sitting (92) (Appendix B). To calculate the score, the Metabolic equivalent task (METs) of the various activities were calculated as: (a) number of days in which vigorous physical activity took place x minutes spent on vigorous activity x 8; (b) number of days

during which moderate physical activity took place x minutes spent on moderate activity x 4; (c) number of days walked x minutes spent walking x 3.3. Total METs were calculated as the sum of Walking, Moderate, and Vigorous MET scores. If total METs were ≤700, the subject was classified as inactive, for scores between 700 and 2519 the subject was classified as moderately active, and for score ≥ 2520 the subject was classified as active.

4.2.4 Definitions

The body mass index (60) was calculated as weight in kg divided by height in metres square (kg/m^2). A "normal BMI" was evaluated when the parameter was ranged between 18.5 and 24.9, while <18.5 included underweight patients, >24.9 overweight and >29.9 obese.

According to modified, revised NCEP-ATP III (Third Report of the National Cholesterol Education Program) criteria (28), diagnosis of metabolic syndrome (MetS) was made when the patient met at least three of the following criteria:

- Triglyceride levels ≥150 mg/dl or drug treatment for hypertriglyceridemia
- High-density lipoprotein (HDL)-C <40 mg/dl in men and <50 mg/dl in women or drug treatment to raise HDL-C levels
- Diabetes mellitus (DM) and treatment for elevated glucose or fasting glucose levels ≥100 mg/dl
- Blood pressure ≥130/85 mm Hg or on antihypertensive drug treatment
- Waist circumference >102 cm in men and >88 cm in women. This latter parameter was substituted for body mass index (60) > 30 as the cut-off point for obesity.

4.2.5 Statistical analysis

Categorical variables were expressed as absolute frequency and percentage and quantitative variables as mean ± standard or median (interquartile range) according to data distribution, after performing the Kolmogorov-Smirnov test for normality.

All statistics were performed using the Statistical Package for Social Sciences (SPSS) program (Chicago, IL, USA).

4.3 Results

Demographic and clinical characteristics prior to HTx and at questionnaires completion of the study cohort are shown in Table 6. Of 143 patients who filled the questionnaires, 115 (80%) were men. The median age at the time of HTx was 54 (47-60) years, with most of them aged between 41 – 59 years (60%). Eighty-one% of patients were from North Italy, while 19% from Centre-South. Thirteen percent of patients had a BMI>30 before HTx and 23% were affected by MetS. After 1 year of follow-up since HTx, the percentage of patients affected by MetS increased to 42%.

Patients filled the questionnaires after a median time of 9 (4-12) years after HTx, the median age at completion was 64 (57-69) years, with 7% of patients having ≤40 years old, 33% between 41 and 59 years old, and 60% ≥60 years old. Median BMI was 27 kg/m^2, range 17-43 kg/m^2, with 3% underweight patients, 26% normal weight, 39% overweight and 32% obese. In total, 71% of patients had a BMI over the normal range.

Table 6. Patients' data at questionnaires completion

	N = 143
Median Age at HTx, years	54 (47-60)
≤40 years, n (%)	19 (13)
41 – 59 years, n (%)	86 (60)
≥60 years, n (%)	38 (27)
Male/Female, n (%)	115 (80) / 28 (20)
Provenience, n (%) **North Italy** **Centre-South Italy**	116 (81) / 27 (19)
Pre-HTx BMI>30, n (%)	18 (13)
Pre-HTx MetS, n (%)	33 (23)
MetS at 1 year f-up	60 (42%)
Median age at completion, years	64 (57-69)
≤40 years, n (%)	10 (7)
41 – 59 years, n (%)	47 (33)
≥60 years, n (%)	86 (60)
Median time from HTx, years	9 (4-12)
Mean Weight, kg	82±19
Median BMI, kg/m^2	27 (17-43)
Underweight, n (%)	4 (3)
Normal weight, n (%)	37 (26)
Overweight, n (%)	56 (39)
Obese, n (%)	46 (32)

BMI = body mass index; HTx = heart transplantation; MetS = metabolic syndrome;

Distribution of the Mediterranean diet adherence score

Table 7 displays the results of the FFQ and IPAQ questionnaires. The median MedDiet score was 4, range 2 – 5, indicating an overall weak adherence to the MedDiet in this patient population. Only 25% of patients had a good adherence, and only 8% of patients had both a good adherence and a normal BMI. IPAQ was filled by 114 (80%) of patients, with a median score of 2395, range 735 – 4290, and 25% of them resulted as physically inactive, 26% sufficiently active and 49% as active. Finally, 13% was the amount of patients with both a good FFQ score, normal BMI and a good IPAQ score.

Comparing patients with or without MetS at 1 year after HTx (Table 8), 38% of patients with MetS were already affected by this condition before HTx. The prevalence of Cardiac Allograft Vasculopathy (CAV) was similar in the two groups, while patients with MetS had, as expected, an higher mean weight (91±19 kg vs. 76±16 kg, $p<0.001$) and median BMI (31 (27 – 33) vs. 25 (23 – 28), $p<0.001$). No difference was found in the median MedDiet score between the two groups, while patients with MetS resulted less physically active (1381 (429 – 3750) vs. 3042 (1030 – 4530), $p=0.026$).

Figure 10 represents a visual distribution of the MedDiet score, clearly shifted towards a low adherence to the MedDiet, with 75.6% of patients having a score <6.

In Figure 11, a visual distribution of the 15 items included in the FFQ is shown. In blue are reported the items not considered for the score calculation, while the other 10 items were expressed with green and red bars, depending on the frequency of consumption. The green bars indicated the interval of frequency of consumption used to calculate the MedDiet score (+1 point). From this analysis it was observed that only half of the patients consumed vegetables and fruits 2 or more times a day (52% and 50% respectively), while even less of them consumed daily whole grain products instead of normal (36% whole grain pasta/rice; 47% whole grain bread). Also extra-virgin olive oil (EVOO) was mostly consumed in an inadequate frequency, with only 24% of patients using it as preferred dressing several times a day. Consumption of red wine at meal in the recommended frequency, assures 1 point in the MedDiet score, although the majority of patients in this study (57%) did not drink wine at all. Red meat consumption was the only item where the recommended frequency was reached by almost all the patients (94%). Finally, Fish, Nuts and Legumes, were the less adequately consumed: only 31%, 30% and 25 % of patients consumed legumes, seafood and nuts, respectively, more than 2 times a week.

Table 7. Patients' questionnaires scores

	N = 143
Median MedDiet score	4 (2-5)
Good adherence, n (%)	35 (25%)
Good adherence + normal BMI, n (%)	11 (8%)
N. patients who filled IPAQ	114 (80%)
Median IPAQ score	2395 (735-4290)
Inactive, n (%)	28 (25%)
Sufficiently active, n (%)	30 (26%)
Active, n (%)	56 (49%)
Good adherence + normal BMI + good IPAQ, n (%)	19 (13%)

BMI = body mass index; MedDiet = Mediterranean diet.

Table 8. Comparison between patients with and without MetS at 1 year after HTx

	No MetS at 1 year f-up (n. 83)	1-year f-up MetS (n.60)	p
Pre-HTx MetS	10 (12)	23 (38)	0.000
Cardiac allograft vasculopathy, n (%)	24 (29)	13 (22)	0.329
Median age at completion, years	63 (57 – 69)	64 (57 – 69)	0.977
Median time from HTx, years	11 (5 – 15)	6 (3 – 10)	0.000
Mean Weight, kg	76±16	91±19	0.000
Median BMI, kg/m^2	25 (23 – 28)	31 (27 – 33)	0.000
Median MedDiet score	4 (2 – 5)	4 (3 – 6)	0.257
Good adherence, n (%)	20 (24)	15 (25)	0.901
Good adherence + normal BMI, n (%)	7 (8)	4 (7)	0.761
Median IPAQ score	3042 (1030 – 4530)	1381 (429 – 3750)	0.026
Good adherence + normal BMI + good IPAQ, n (%)	12 (15)	7 (12)	0.627

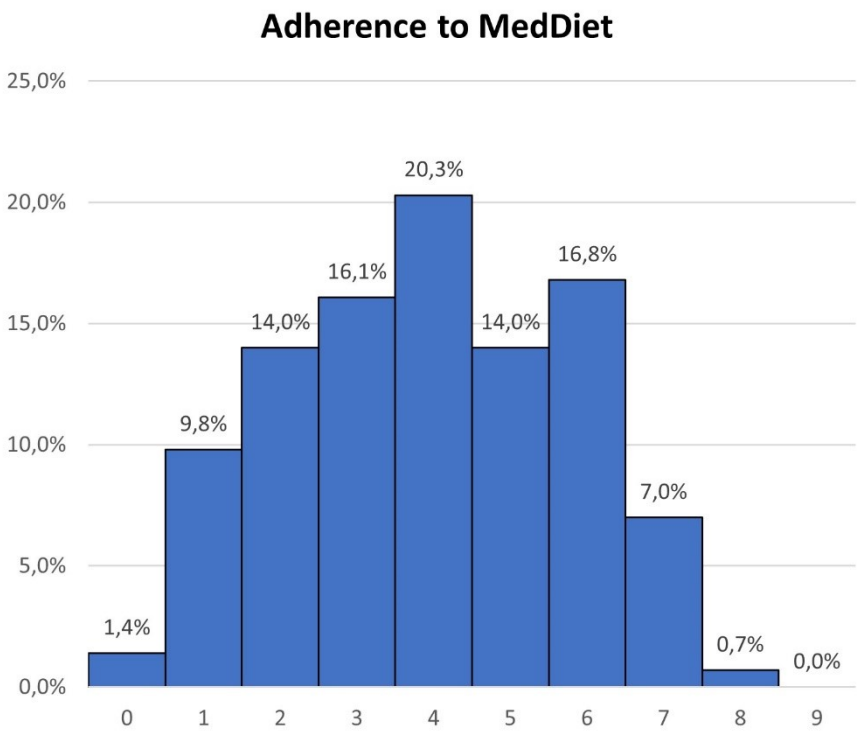

Figure 10. Visual distribution of patients' adherence score to MedDiet.

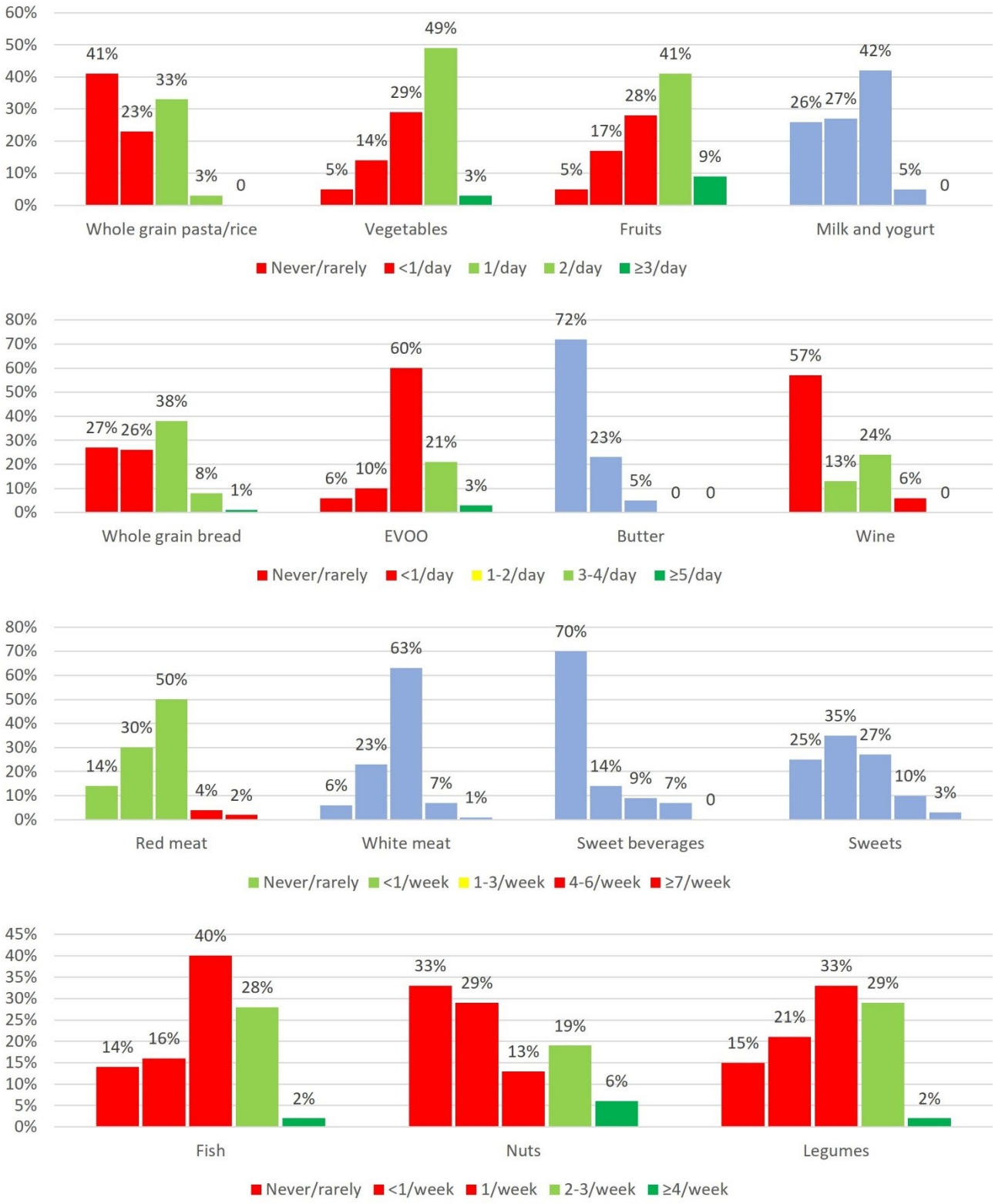

Figure 11. Visual distribution of the 15 FFQ items in the study population.

4.4 Discussion

Among all dietary patterns, the Mediterranean diet has proved to be the most effective in reducing the cardiovascular risk factors (65, 66), as well as in reversing and limiting the MetS development in general adult population (68, 69). Since MetS frequently affects transplanted patients (46), mostly due to the immunosuppressive therapy, a structured dietary approach, following the MedDiet indications, could be of paramount importance in controlling all the MetS related factors. However, no specific guidelines on the correct dietary approach to adopt over the long-term period after HTx have been published to date. Moreover, the current evidence regarding MedDiet intervention on HTx patients is limited to only two studies (59, 61).

Since the prevalence of MetS turned out to be really high in HTx patients of our centre, as described in Aim 1, we intended to analyse the level of adherence to the MedDiet in these patients. In our sample of 143 HTx patients investigated through a validated FFQ (90), we found a median MedDiet score of 4 (range 2-5), with 75.6% of patients scored <6. Since 6 was chosen as the cut-off point to discriminate a good adherence, in line with other studies (90, 91), 3 patients out of 4 resulted weakly adherent to MedDiet. Moreover, the low adherence was similar among patients with or without MetS at the first year after HTx.

The IPAQ questionnaire (92), instead, revealed that about half of the patients (51%) resulted physically inactive or only sufficiently active, and that patients with MetS at 1 year of follow-up were more sedentary. This last result could be linked to the higher BMI that characterises these patients, making physical activity more challenging and creating a dangerous vicious circle.

As a matter of fact, Lee at al. reported that adherence to both physical activity and dietary guidelines is associated with a lower odds of MetS, as long as a lower risk to develop MetS prospectively, in 2379 Framingham Heart Study participants (mean age 47 years; 54.4% women) (53). However, no study have been published so far investigating adherence to both physical activity and dietary recommendations in HTx patients, even if we can presume a similar effect. Indeed, the weak adherence to both MedDiet and physical activity observed in this series was accompanied with a high percentage of overweight and obese patients (71%).

Focusing more in depth about the various items included in the FFQ, we effectively noted that the patients did not consume with a properly frequency all those foods related to the beneficial effects of MedDiet. For instance, only half of the patients consumed the adequately daily serving of fruits and vegetables, the principal pillar of MedDiet and correlated with its anti-inflammatory action, as previously described by many studies (66, 67, 69, 71). They also did not reach the recommended

frequency of consumption of EVOO and nuts, two foods that have been correlated, in the PREDIMED study, to a reduced rate of cardiovascular events and regression of MetS (68). Moreover, patients in this study population did not habitually consumed whole grain products and legumes, which are known food rich in non-digestible carbohydrate, or fibres. As described previously, fibres has many beneficial effects on health, including anti-inflammatory effects (82). Fibres act also in controlling the post-prandial glycaemia, particularly important in HTx patients, who are frequently affected by insulin resistance or diabetes (83, 85, 86). Finally, also fish and seafood were consumed below the recommended frequency. These foods are the principal source of omega-3 polyunsaturated fatty acids, associated to cardio-protective effects (42), body weight reduction and metabolic profile improvement (31), and anti-inflammatory activity (87).

The PREDIMED study showed a 2-point increase in the MedDiet score in a non-transplant population to be associated with a 14% reduction of all-cause mortality (93), and a 1-point increase among a population at high risk of cardiovascular diseases to be associated with a 18% reduction of myocardial infarction (94). Although MedDiet adherence was assessed differently in these studies, it appears of huge importance to make an effort in improving dietary habits of HTx patients towards a MedDiet-dietary pattern.

A previous study by Entwistle et al. demonstrated that a structured dietary education among 39 clinically stable HTx and lung-transplanted patients, exerted for 12 months, resulted in an increased adherence to the MedDiet, as well as changes in many cardiovascular risk factors, such as a decreased BMI, fasting glucose and total cholesterol (59, 60). This experience proved that an implementation in nutritional intervention in HTx patients is feasible and effective, and may lead to many beneficial changes in the metabolic profile of these patients. Thus, the third aim of this research was to evaluate the impact of a structured personalized nutritional education in a group of cardiac transplanted patients from our centre.

5 Experimental part: Aim 3

Evaluation of a structured nutritional education in cardiac transplanted patients: pilot study

5.1 Aim

Evaluate the impact of a structured personalized nutritional education in a group of cardiac transplanted patients, compared to a control group

a. Evaluate change in adherence to the Mediterranean diet in the two groups
b. Determine change in the clinical, anthropometric, body composition and blood parameters in the two groups
c. Evaluate change in the dietary habits of cardiac transplanted patients who received the structured personalized nutritional education

5.2 Methods

5.2.1 Patient population

Cardiac transplanted (HTx) patients followed by the Transplant Ambulatory at University Hospital of Udine who accepted to participate at the study from October 2020 to December 2021 were enrolled.
Exclusion criteria were:
- patients with a follow-up <12 months after heart transplantation
- patients younger than 18 years old
- patients with acute rejection, inflammatory bowel disease, coeliac disease, alimentary allergies or intolerances, eating behaviour disorders.

5.2.2 Study design

In this experimental clinical study, patients enrolled were randomized in an intervention and a control group (Figure 12).
The intervention group pursued a structured nutritional education composed of 3 steps: meeting 1 (baseline), meeting 2 (at 6 months), and final meeting (at 12 months).
At baseline, patients were invited to compile a food diary, a food frequency questionnaire (FFQ) and a questionnaire to evaluate the physical activity rate (IPAQ). Body composition was evaluated and blood, clinical and anthropometrics parameters collected. All these data were used to estimate the

adequate energy requirements of patients, personalize the nutritional education and reduce the underlined critical comorbidities.

The food diary, the FFQ and the IPAQ were proposed again at meeting 2 and 3 to assess the progress of the nutritional education. Body composition and anthropometric parameters were evaluated at all meetings.

The control group, instead, pursued the standard clinical follow-up at the Heart Transplant ambulatory. Besides, in the context of the regular cardiologic monitoring, at baseline and at the next cardiologic visit the FFQ, the IPAQ, body composition and anthropometrics parameters were collected.

At baseline, patients from control group received general nutritional advices based on the Guidelines for a healthy nutrition in the Italian population by CREA (95) (Appendix C).

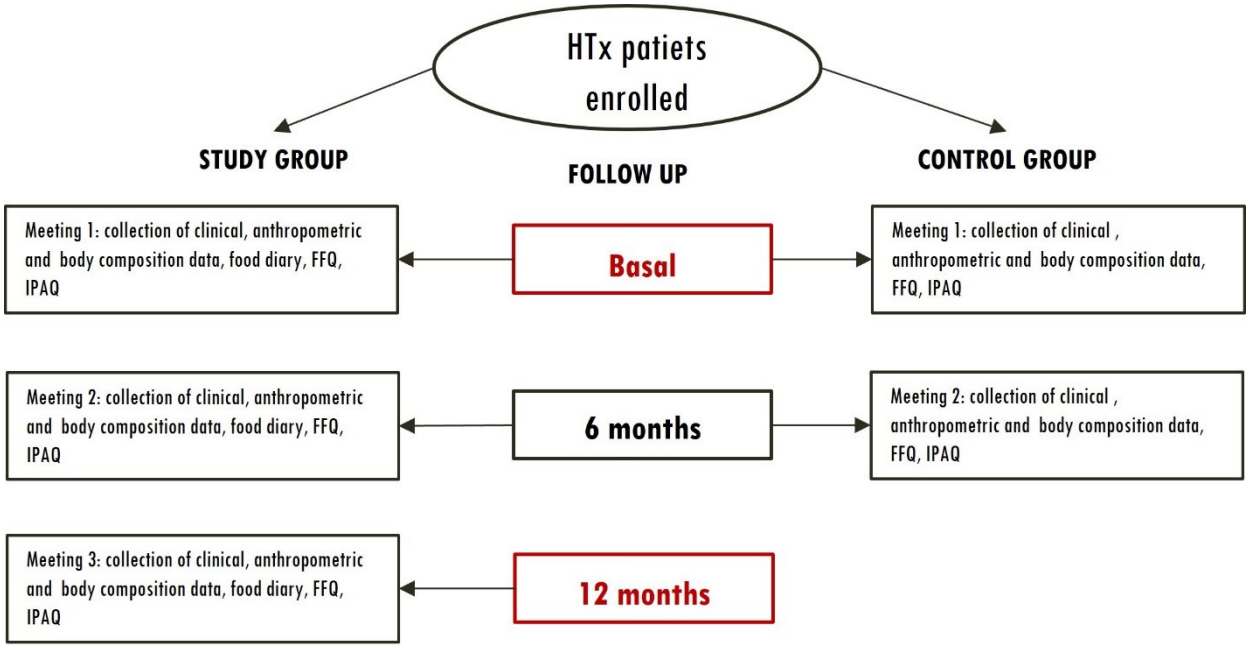

Figure 12. Study design.

5.2.3 Food diary

The food diary was designed to collect all the specific foods, with related portions, consumed by the patients during 4 days, 2 week days and 2 weekend days (Appendix D). It is a simple and useful tool to evaluate both the baseline dietary habits of patients and any eventual change during the nutritional education program. The food diaries were analysed with the software Microdiet (version 4.4.1; Downlee Systems Ltd, High Peak, UK), in order to evaluate the following nutritional elements:

- Total carbohydrates, sugars, fibres
- Total proteins
- Total fat, total saturated fatty acids (SFAs), total mono and poly-unsaturated fatty acids (MUFAs and PUFAs), oleic acid (18:1), linoleic acid (18:2), α-linoleic acid (18:3), arachidonic acid (20:4), eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6) and cholesterol
- Minerals: sodium (Na), potassium (K), calcium (Ca), iron, zinc, magnesium (Mg), phosphorus (P), copper (Cu), selenium (Se), chloride (Chlor), manganese (Mn) and iodine
- Vitamins: A (retinol-equivalent), C, E (α-tocopherol equivalent), D, K, thiamine (B1), riboflavin (B2), B6, B12, niacin (B3), biotin (B8), pantothenic acid (B5) and folic acid (B9)

The 2014 Reference Assumption Levels of Nutrients and energy for Italian population (LARN 2014) were used to compare dietary intakes of patients with reference intakes(96).

5.2.4 FFQ and IPAQ questionnaires

The FFQ and IPAQ questionnaires used in this study were the same described in Experimental part: Aim 2. For the FFQ was used the Mediterranean diet (MedDiet) adherence questionnaire by Gnagnarella et al (90) (Appendix A), while the IPAQ was the one proposed by Hangströmer and colleagues (92) (Appendix B).

5.2.5 Data collection

Clinical data

For all the cardiac transplanted patients enrolled, demographic and clinical data were collected from clinical informatic system and patients chart, including:

- Clinical history pre and post-HTx and comorbidities
- Complications during the long-term outcome post-HTx (acute rejection episodes, infections, re-hospitalizations, etc.)
- Pharmacological therapy (immunosuppressive treatment, anti-hypertensive drugs, statins, etc.)
- Echocardiographic parameters and blood pressure

Blood parameters

Data on blood parameters were collected from routinely blood analysis of patients, and they included:

- Full blood count (white blood cells (WBC), haemoglobin (25))
- Fasting glucose
- Lipid profile (total cholesterol, LDL and HDL cholesterol, triglyceride)
- Renal function parameters (serum creatinine, eGFR calculated through EPI-CKD equation)
- Liver function parameters (SGOT, SGP, Bilirubin)

Anthropometric and body composition parameters

Anthropometric and body composition parameters were collected at every scheduled meeting. The anthropometric data were measured by a nutritionist, and they included:

- Weight and height
- Waist and hips circumferences

Body composition data were obtained using Nutrilab Bioimpedance device (Akern 2016 with Biatrodes, Akern electrodes):

- Body Cell Mass (BCM): the metabolically active part of the organism that carries out all the functional work, the "engine" of the body in which all the main metabolic processes occur, from oxygen consumption, glucose oxidation, to protein synthesis. It is the living and active part of the organism, a compartment that the human body should be provided with in abundance.
- Total Body Water (TBW): the main component of our organism: the total body fluids present in the body. It is expressed as a percentage of body weight. This compartment tends to decrease with age, due to physiological loss of FFM.
- Extra-cellular Water (ECW): the fluids outside the cells. It's located mainly in the space between the cells, at internal blood vessels, lymphatic tissues and spinal fluid.
- Intra-cellular Water (ICW): the fluids inside the cells.
- Fat Mass (FM): all the body fat mass. A good physical form assumes a FM value of 15-23% compared to body weight, depending on the age of subject.
- Fat-Free Mass (FFM): the compartment that contains everything that's not fat mass: it includes the skeleton, about 73% of body fluids, muscles, skin, and organs. Good physical fitness assumes an FFM value of 77-85% compared to body weight, depending on the age of the subject.

5.2.6 Mediterranean dietary education

During the scheduled meetings, patients of the intervention group received specific information and nutritional advices from a trained nutritionist, who encouraged the adherence to a Mediterranean dietary pattern. After the meeting, the nutritionist elaborated and gave to all the patients a written report with personalized nutritional indications, including examples on how to compose correctly daily meals.

5.2.7 Definitions

The body mass index (60) was calculated as weight in kg divided by height in metres square (kg/m^2). A "normal BMI" was evaluated when the parameter was ranged between 18.5 and 24.9, while <18.5 included underweight patients, >24.9 overweight and >29.9 obese.

According to modified, revised NCEP-ATP III (Third Report of the National Cholesterol Education Program) criteria (28), diagnosis of metabolic syndrome (MetS) was made when the patient met at least three of the following criteria:

- Triglyceride levels ≥150 mg/dl or drug treatment for hypertriglyceridemia
- High-density lipoprotein (HDL)-C < 40 mg/dl in men and <50 mg/dl in women or drug treatment to raise HDL-C levels
- Diabetes mellitus (DM) and treatment for elevated glucose or fasting glucose levels ≥100 mg/dl
- Blood pressure ≥130/85 mm Hg or on antihypertensive drug treatment
- Waist circumference >102 cm in men and >88 cm in women.

Insulin resistance (IR) was indirectly calculated by measuring the TG/HDL ratio, as previously reported in the study by Biadi et al. (49). A TG/HDL of 3.0 or more was considered as an index of IR.

The estimate glomerular filtration rate (eGFR) was calculated through EPI-CKD equations.

5.2.8 Sample size

To determine the sample size, it was considered the variation of total cholesterol level in the blood as a principal effect of efficiency of the nutritional education program. Considering an effect size of 0.5, it was calculated a sample size of 64 patients in each group, intervention and control group, to obtain a statistical power of 80%, with an α-error of 0.5. However, as specified in the "study limitations"

part, the study was performed during the COVID pandemic, so we were not able to reach the adequate number of patients planned.

5.2.9 Statistical analysis

Categorical variables were expressed as absolute frequency and percentage, quantitative variables as mean ± standard or median (interquartile range) according to data distribution, after performing the Shapiro-Wilk test for normality.

Intervention group vs control group comparison

Data of Intervention and Control group were compared at the following timepoints:

1. At baseline
2. Intermediate: Intervention group meeting 2 vs. Control group final meeting

The choice was made in order to both show any progressive change in the collected parameters for the Intervention group and include as many patients as possible in the analysis, not disposing of adequate time to reach the final meeting for all the participants enrolled.

At baseline, were included all patients of both intervention and control group who reached the meeting 2, in order to ensure the comparability between the two groups.

Comparisons of continuous variables were made with Student's t-test for independent samples or the Mann–Whitney U test if the variables did not fit a normal distribution. Differences between percentages were compared using Pearson's chi-squared test with Fisher's correction when the number of expected values was less than five.

Pre- and post- intra-group comparison

A comparison of data at the various timepoints were made for each group as followed:

1. Control group: comparison between baseline and final data to evaluate any change occurred after general nutritional advices, in the absence of a structured dietary education
2. Intervention group: comparison between 3 timepoints to evaluate any progressive change
    a. Baseline vs. intermediate (meeting 2)
    b. Intermediate vs. final (meeting 3)
    c. Baseline vs. final

Comparisons of continuous variables were made with Student's t-test for paired samples or the Wilcoxon Signed Rank Test if the variables did not fit a normal distribution. Differences between percentages were compared using Pearson's chi-squared test with Fisher's correction when the number of expected values was less than five.

All statistics were performed using the Statistical Package for Social Sciences (SPSS) program (Chicago, IL, USA).

5.3 Results

5.3.1 Study flow-chart

Between October 2020 and December 2021, we were able to enrol 28 HTx patients, 15 in the intervention group and 13 in the control group. Four dropouts occurred in the intervention group, mostly because patients were scared to return in hospital for the scheduled nutritional meetings due to COVID-19 pandemic, as specified in the "study limitations" part. For this reason, half of the patients of the intervention group were enrolled after getting vaccinated, from March 2021, thus only 11 and 7 patients reached the meeting 2 and 3, respectively. In the control group 1 dropout was observed, a patient died for SARS-CoV-2 infection (Figure 13).

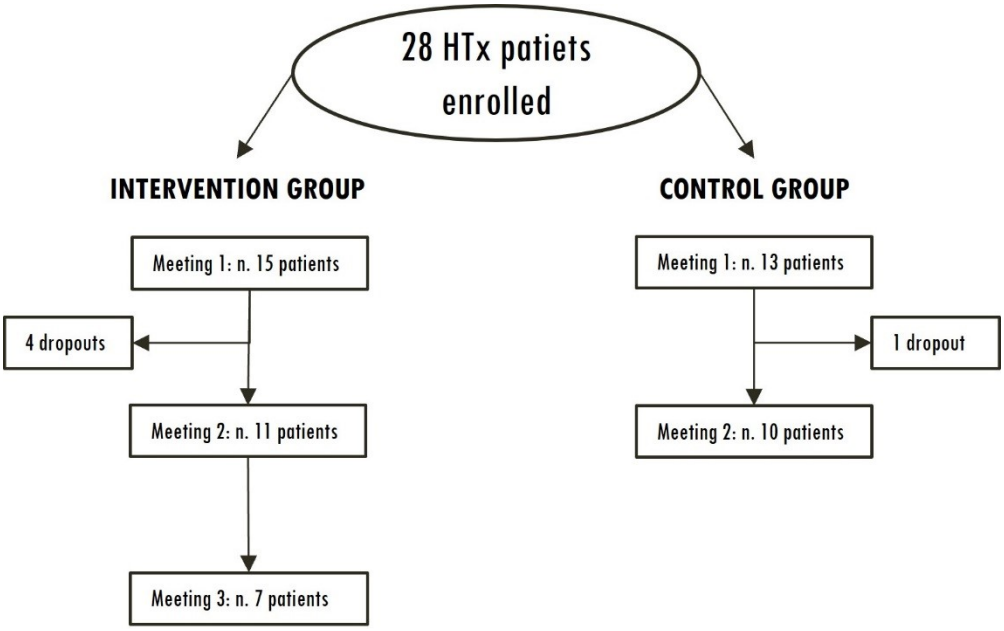

Figure 13. Study flow-chart of cardiac transplanted patients involvement for intervention and control group.

5.3.2 Baseline patients characteristics: Intervention vs. Control group

Baseline patients characteristics of Intervention and Control group are presented in Table 9. Eleven patients were included in the Intervention group and 10 in the Control group. Patients from control group were tendentially older (65±7 years vs. 57±14 years, p=0.078) and less recently transplanted (10, range 5-12 years vs. 4, range 3-10 years, p=0.085), while there was a similar percentage of female participants (9% vs. 20% for intervention and control group respectively, p=0.462). Patients from both groups showed similar rates of pre-HTx MetS prevalence and related criteria. Immunosuppressive therapy was also similar between the two groups, apart for 3 (27%) patients of intervention group taking Tacrolimus vs. none of the control group, (p=0.124). Moreover, while patients of both groups usually took hypertension (HTN) treatments (82% vs. 80% for intervention and control group, respectively, p=0.669), all patients of intervention group took statins vs. 6 (60%) of control group (p=0.035).

Table 9. Baseline patients characteristics of intervention and control group.

Baseline	Intervention group (n.11)	Control group (n.10)	p
Clinical data			
Mean age, years	57±14	65±7	0.078
Male/Female, n (%)	10 (91) / 1 (9)	8 (80) / 2 (20)	0.462
Median time from HTx, years	4 (3-10)	10 (5-12)	0.085
Former smoker, n (%)	6 (55)	5 (50)	0.590
Pre-HTx BMI>30, n (%)	1 (9)	2 (20)	0.462
Pre-HTx TGL≥150 mg/dl, n (%)	2 (18)	3 (30)	0.450
Pre-HTx DM or GLU≥100 mg/dL, n (%)	2 (18)	1 (10)	0.538
Pre-HTx HTN, n (%)	1 (9)	1 (10)	0.738
Pre-HTx HDL<40 or <50, n (%)	4 (36)	2 (20)	0.367
Pre-HTx MetS, n (%)	1 (9)	0	0.524
Pharmacological therapy			
Cyclosporine, n (%)	7 (64)	8 (80)	0.367
Everolimus, n (%)	4 (36)	4 (40)	0.608
Tacrolimus, n (%)	3 (27)	0	0.124
MMF, n (%)	7 (64)	6 (60)	0.608
Corticosteroids, n (%)	2 (18)	2 (20)	0.669
HTN treatment, n (%)	9 (82)	8 (80)	0.669
Statins, n (%)	11 (100)	6 (60)	**0.035**

BMI = body mass index; DM = diabetes mellitus; GLU = glucose; HDL = high-density lipoprotein-cholesterol; HTN = hypertension; HTx = heart transplantation; MetS = metabolic syndrome; MMF = mycophenolate; TGL= triglyceride.

Anthropometric, body composition and echocardiographic data

In Table 10 are showed the anthropometric, body composition and echocardiographic data in intervention vs. control group at baseline. The two groups were similar in all considered parameters, with a BMI shifted towards overweight (24.6±4.5 kg/m^2 vs. 25.7±4.0 kg/m^2, p=0.575, for intervention vs. control group, respectively) and a mean waist circumference (WC) of 93.5±16.7 cm vs. 93.4±16.4 cm (p=0.994) for intervention vs. control group, respectively.

In both intervention and control group FM% was over the recommended range (23.1±7.1% vs. 23.5±7.4%, p=0.908), with a consequent under range FFM% (76.9±7.1% vs. 76.4±7.4%, p=0.908).

Blood pressure was tendentially higher in control group, with a mean systolic blood pressure (sBP) of 144±10 mmHg vs. 132±18 mmHg (p=0.165), and a mean diastolic blood (dBP) of 90±13 mmHg vs. 81±11 mmHg (p=0.162). Both left ventricular ejection fraction (LVEF) and systolic pulmonary artery pressure (sPAP) were comparable (67±6% vs. 70±4%, p=0.199 and 28±6 mmHg vs. 26±3 mmHg, p=0.415 for intervention vs. control group, respectively).

Table 10. Baseline anthropometric, body composition and echocardiographic data in intervention vs. control group

Baseline	Intervention group (n.11)	Control group (n.10)	p
Anthropometric and body composition data			
Mean weight, kg	77.6±18.6	75.4±17.3	0.780
Mean BMI, kg/m^2	24.6±4.5	25.7±4.0	0.575
Mean WC, cm	93.5±16.7	93.4±16.4	0.994
Mean HC, cm	94.0±8.8	98.7±7.8	0.216
Mean WC/HC	0.99±0.13	0.94±0.11	0.414
Mean BCM%	52.0±4.7	48.5±6.5	0.187
Mean TBW%	58.6±5.4	60±6.9	0.632
Mean ECW%	47.4±4.4	50.7±6.2	0.185
Mean ICW%	52.6±4.4	49.3±6.1	0.185
Mean FM%	23.1±7.1	23.5±7.4	0.908
Mean FFM%	76.9±7.1	76.4±7.4	0.908
Echocardiographic data			
Mean sBP, mmHg	132±18	144±10	0.165
Mean dBP, mmHg	81±11	90±13	0.162
Mean LVEF, %	67±6	70±4	0.199
Mean sPAP, mmHg	28±6	26±3	0.415

BCM = body cell mass; BMI = body mass index; dBP = diastolic blood pressure; ECW = extra-cellular water; rate; FM = fat mass; FFM = fat free mass; HC = hip circumference; ICW = intra-cellular water; LVEF = left ventricular ejection fraction; sBP = systolic blood pressure; sPAP = systolic pulmonary artery pressure; TBW = total body water; WC = waist circumference.

Blood parameters

Table 11 displays the blood parameters comparison in intervention and control group at baseline. The two groups were comparable for all the considered parameters, including fasting glucose (GLU, mean 89±14 mg/dL vs. 91±15 mg/dL, p=0.783 for intervention vs. control group, respectively), and lipid profile (mean total cholesterol 169±32 mg/dL vs. 182±45 mg/dL, p=0.457, mean HDL-Cholesterol, 60±15 mg/dL vs. 60±18 mg/dL, p=0.993, mean LDL-C 90±25 mg/dL vs. 102±29 mg/dL, p=0.334 and median triglyceride (TGL) 96, range 53 – 141 mg/dL vs. 91, range 74 – 121, p=0.557 for intervention vs. control group, respectively). IR was present in 18% of patients of the intervention group compared to 10% in control group (p=0.538).

Table 11. Baseline blood parameters in intervention vs. control group

Baseline	Intervention group (n.11)	Control group (n.10)	p
Blood parameters			
Mean Hb, g/dL	14.2±1.5	14.1±1.4	0.820
Mean WBC, x10^3/µL	6.6±1.8	6.0±1.5	0.500
Mean GLU, mg/dL	89±14	91±15	0.783
Median Serum Creatinine, mg/dL	1.13 (1.05 – 1.43)	1.16 (0.98 – 1.52)	0.809
Median eGFR, mL/min/1.73mq	67 (61 – 77)	63 (47 – 74)	0.315
Mean SGOT, UI/L	23±8	22±6	0.726
Median SGP, UI/L	18 (14 – 24)	19 (16 – 26)	0.863
Mean Total Bilirubin, mg/dL	0.94±0.41	0.85±0.42	0.629
Mean Total Cholesterol, mg/dL	169±32	182±45	0.457
Mean LDL-C, mg/dL	90±25	102±29	0.334
Mean HDL-C, mg/dL	60±15	60±18	0.993
Median TGL, mg/dL	96 (53 – 141)	91 (74 – 121)	0.557
Mean TGL/HDL ratio	1.7±1.0	1.8±0.8	0.883
IR	2 (18%)	1 (10%)	0.538
Mean Serum Albumin, g/L	45±3	43±3	0.091
Mean Uric Acid, mg/dL	5.6±0.9	5.4±1.0	0.580
Median Serum Iron, µg/dL	84 (72 – 94)	67 (59 – 85)	0.114

eGFR = estimated glomerular filtration rate; GLU = glucose; Hb = haemoglobin; HDL-C = high-density lipoprotein-cholesterol; IR = insulin resistance; LDL-C = low-density lipoprotein-cholesterol; SGOT = serum glutamic oxaloacetic transaminase; SGP = serum glutamyl pyruvic transaminase; TGL= triglyceride.

Metabolic syndrome prevalence

At baseline, 46% of participants from intervention group resulted affected by MetS compared to 20% in the control group (p=0.221). There was a tendency in intervention group patients to have more hypertriglyceridemia (100% vs. 70% p=0.090), while the two groups were comparable for the other MetS criteria (Table 12).

Table 12. Metabolic syndrome prevalence at baseline in intervention vs. control group

Baseline	Intervention group (n.11)	Control group (n.10)	p
Metabolic syndrome			
WC>102 or >88, n (%)	4 (36)	4 (40)	0.608
TGL≥150 or treatment mg/dl, n (%)	11 (100)	7 (70)	0.090
DM or GLU≥100 mg/dL, n (%)	3 (27)	2 (20)	0.550
HTN or HTN treatment, n (%)	10 (91)	9 (90)	0.738
HDL<40 or <50, n (%)	0	1 (10)	0.476
MetS, n (%)	5 (46)	2 (20)	0.221

BMI = body mass index; DM = diabetes mellitus; HDL = high-density lipoprotein-cholesterol; HTN = hypertension; MetS = metabolic syndrome; TGL = triglyceride.

5.3.3 Intermediate patients characteristics: Intervention vs. Control group

Eleven patients of intervention group reached the meeting 2 after a median of 5.8 (4.7 – 6.3) months, while patients of control group reached their final meeting after a median of 6.1 (6.0 – 10.5) months (p=0.099). Pharmacological therapy remained almost the same and comparable between the two groups, with 3 (27%) patients of intervention group taking Tacrolimus vs. none of the control group (p=0.124) and 7 (70%) of them taking statins vs. all (11, 100%) patients of intervention group (p=0.090) (Table 13).

Table 13. Intermediate patients characteristics of intervention and control group.

Intermediate	**Intervention group (n.11)**	**Control group (n.10)**	**p**
Median time from baseline, months	5.8 (4.7 – 6.3)	6.1 (6.0 – 10.5)	0.099
Pharmacological therapy			
Cyclosporine, n (%)	7 (64)	8 (80)	0.367
Everolimus, n (%)	4 (36)	4 (40)	0.608
Tacrolimus, n (%)	3 (27)	0	0.124
MMF, n (%)	7 (64)	6 (60)	0.608
Corticosteroids, n (%)	2 (18)	2 (20)	0.669
HTN treatment, n (%)	9 (82)	7 (70)	0.450
Statins, n (%)	11 (100)	7 (70)	0.090

HTN= hypertension; MMF = mycophenolate.

Anthropometric, body composition and echocardiographic data

Table 14 shows the comparison of anthropometric, body composition and echocardiographic data between intervention and control group at the intermediate timepoint. Both BMI and circumferences remained comparable in the two groups, as well as the body composition parameters. However, a positive shift towards a lower FM% resulted for both groups (18.4±7.7% vs. 15.9±7.3%, p=0.559 for intervention vs. control group, respectively), reaching the recommended range, with a consequent higher FFM% (81.6±7.7% vs. 84.1±7.3%, p=0.559 for intervention vs. control group, respectively). Blood pressure resulted significantly lower in intervention group (median sPB 130, range 120 – 130 mmHg vs. 145, range 130 – 147, p=0.020 and median dBP 80, range 75 – 80 mmHg vs. 86, range 83 – 90, p=0.004), while LVEF and sPAP remained comparable.

Table 14. Intermediate anthropometric, body composition and echocardiographic data in intervention vs. control group

Intermediate	**Intervention group (n.11)**	**Control group (n.10)**	**p**
Anthropometric and body composition data			
Mean weight, kg	77.3±18.0	76.3±19.0	0.904
Mean BMI, kg/m^2	24.5±4.5	26.0±4.6	0.451
Mean WC, cm	91.4±15.3	90.3±14.2	0.880
Mean HC, cm	94.3±7.9	94.9±6.6	0.849
Mean WC/HC	0.96±0.12	0.95±0.12	0.808
Mean BCM%	51.8±3.3	52.7±3.5	0.611
Mean TBW%	61.5±5.3	62.3±5.8	0.790
Mean ECW%	47.6±3.1	46.7±3.2	0.609
Median ICW%	52.4±3.1	53.3±3.2	0.609
Mean FM%	18.4±7.7	15.9±7.3	0.559
Mean FFM%	81.6±7.7	84.1±7.3	0.559
Echocardiographic data			
Median sPB, mmHg	130 (120 – 130)	145 (130 – 147)	**0.020**
Median dBP, mmHg	80 (75 – 80)	86 (83 – 90)	**0.004**
Mean LVEF, %	68±7	65±5	0.245
Mean sPAP, mmHg	31±5	26±5	0.122

BCM = body cell mass; BMI = body mass index; dBP = diastolic blood pressure; ECW = extra-cellular water; rate; FM = fat mass; FFM = fat free mass; HC = hips circumference; ICW = intra-cellular water; LVEF = left ventricular ejection fraction; sBP = systolic blood pressure; sPAP = systolic pulmonary artery pressure; TBW = total body water; WC = waist circumference.

Blood parameters

At intermediate timepoint, blood parameters remained comparable between intervention and control group, apart from the median eGFR, which resulted significantly lower in the intervention group (61, range 53 – 65 mL/min/1.73mq vs. 70, range 64 – 77 mL/min/1.73mq, p=0.043). However, a tendency to lower level of mean GLU (84±10 mg/dL vs. 94±18 mg/dL, p=0.133) and TGL was reported for intervention group (87±38 mg/dL vs. 113±41 mg/dL, p=0.153) (Table 15).

Table 15. Intermediate blood parameters in intervention vs. control group

Intermediate	Intervention group (n.11)	Control group (n.10)	p
Blood parameters			
Median Hb, g/dL	14.3 (13.0 – 15.7)	14.1 (13.6 – 14.8)	0.918
Mean WBC, x10^3/µL	6.3±1.5	5.9±1.3	0.481
Mean GLU, mg/dL	84±10	94±18	0.133
Median Serum Creatinine, mg/dL	1.09 (1.07 – 1.26)	1.17 (1.07 – 1.38)	0.512
Median eGFR, mL/min/1.73mq	70 (64 – 77)	61 (53 – 65)	**0.043**
Median SGOT, UI/L	20 (18 – 28)	22 (21 – 30)	0.705
Median SGP, UI/L	19 (14 – 21)	21 (16 – 24)	0.387
Mean Total Bilirubin, mg/dL	0.96±0.39	0.78±0.34	0.276
Mean Total Cholesterol, mg/dL	185±54	180±45	0.842
Median LDL-C, mg/dL	102 (72 – 128)	95 (87 – 118)	0.705
Mean HDL-C, mg/dL	60±12	58±18	0.721
Mean TGL, mg/dL	87±38	113±41	0.153
Median TGL/HDL ratio	1.7 (0.6 – 2.1)	1.9 (1.5 – 2.3)	0.223
IR	1 (9%)	1 (10%)	0.738
Mean Serum Albumin, g/L	46±3	43±3	0.064
Mean Uric Acid, mg/dL	5.7±0.9	5.7±1.1	0.929
Mean Serum Iron, µg/dL	79±18	90±31	0.364

eGFR = estimated glomerular filtration rate; GLU = glucose; Hb = haemoglobin; HDL-C = high-density lipoprotein-cholesterol; IR = insulin resistance; LDL-C = low-density lipoprotein-cholesterol; SGOT = serum glutamic oxaloacetic transaminase; SGP = serum glutamyl pyruvic transaminase; TGL= triglyceride.

5.3.4 Mediterranean diet adherence

Table 16 displays the results from the FFQ used to calculate the adherence to the MedDiet. At baseline, the score was comparable in both intervention and control group (4±2 vs. 4±2, p=0.918). However, in intervention group the score progressively increased, reaching a mean of 5±2 vs. 4±1 (p=0.169) at intermediate timepoint.

Table 16. Results from the FFQ to calculate Mediterranean Diet Adherence.

FFQ	**Intervention group (n.11)**	**Control group (n.10)**	**p**
Baseline mean score	4±2	4±2	0.918
	Intervention group (n.11)	**Control group (n.10)**	
Intermediate mean score	5±2	4±1	0.169

5.3.5 Control group baseline vs. final data comparison

The comparison of overall data from baseline to final timepoints of control group are showed in Table 17. Starting with pharmacological therapy, immunosuppressive treatment was not changed during the study period, while one more patient started taking statins (6 vs. 7, p=0.500).

Mean weight and BMI remained similar, with a tendency to overweight (mean BMI 25.7±4.0 kg/m^2 vs. 26.0±4.6 kg/m^2, p=0.275), as well as mean waist and hips circumferences. Body composition data showed a tendency to a lower mean FM% (26.0±4.7% vs. 17.3±2.0%, p=0.056), with a consequent higher mean FFM% (74.0±4.7% vs. 82.7±2.0 %, p=0.056). Blood pressure values and sPAP remained comparable, while mean LVEF resulted significantly decreased over the study period (71±3% vs. 66±5%, p=0.034).

Blood parameters remained comparable at the two timepoints, including mean fasting glucose (91±15 mg/dL vs. 94±18 mg/dL, p=0.272) and lipid profile (mean total cholesterol 182±45 mg/dL vs. 180±45 mg/dL, p=0.761; mean HDL-C, 60±18 mg/dL vs. 58±18 mg/dL, p=0.791; mean LDL-C, 102±29 mg/dL vs. 100±36 mg/dL, p=0.198; median TGL 91, range 74 - 121 mg/dL vs. 103, range 94 – 117, p=0.610).

As regard questionnaires, both mean FFQ score (4±2 vs. 4±1, p=1.000) and median IPAQ score (4290, range 1680 – 9540 vs. 4620, range 3015 – 8445, p=0.398) remained comparable.

Metabolic syndrome and related criteria prevalence did not change significantly, although, apart for WC, one more patient met each of MetS related criteria and MetS itself.

Table 17. Overall baseline and final data comparison in the control group.

Control group (n.10)	Baseline	Final	p
Pharmacological therapy			
Cyclosporine	8 (80%)	8 (80%)	0.709
Everolimus	4 (40%)	4 (40%)	0.675
Tacrolimus	0	0	
MMF	6 (60%)	6 (60%)	0.675
Corticosteroids	2 (20%)	2 (20%)	0.709
HTN treatment	8 (80%)	7 (70%)	0.500
Statins	6 (60%)	7 (70%)	0.500
Median statin dose	20 (20 – 40)	20 (20 - 80)	0.317
Anthropometric and body composition data			
Mean weight, kg	75.4±17.3	76.3±19.0	0.355
Mean BMI, kg/m^2	25.7±4.0	26.0±4.6	0.275
Mean WC, cm	93.4±16.4	90.3±14.2	0.054
Mean HC, cm	98.7±7.8	94.9±6.6	0.405
Mean WC/HC	0.94±0.11	0.96±0.12	0.251
Mean BCM%	50.7±5.8	53.6±2.6	0.381
Mean TBW%	59.2±3.7	61.0±1.6	0.397
Mean ECW%	48.6±5.4	45.9±2.4	0.382
Mean ICW%	51.4±5.4	54.1±2.4	0.382
Mean FM%	26.0±4.7	17.3±2.0	0.056
Mean FFM%	74.0±4.7	82.7±2.0	0.056
Echocardiographic data			
Mean sPB, mmHg	147±9	136±9	0.162
Mean dBP, mmHg	89±13	86±3	0.540
Mean LVEF, %	71±3	66±5	0.034
Mean sPAP	24±2	24±3	0.591
Blood parameters			
Median Hb, g/dL	13.8 (13.5 – 15.0)	14.1 (13.6 – 14.8)	0.184
Mean WBC, x10^3/µL	6.0±1.5	5.86±1.30	0.528
Mean GLU, mg/dL	91±15	94±18	0.272

Mean Serum Creatinine, mg/dL	1.24±0.28	1.20±0.18	0.377
Mean eGFR, mL/min/1.73mq	60±14	61±9	0.841
Mean SGOT UI/L	22±6	24±6	0.413
Median SGP, UI/L	19 (16 – 26)	21 (16 – 24)	0.343
Mean total Bilirubin, mg/dL	0.85±0.42	0.78±0.34	0.482
Mean Total Cholesterol, mg/dL	182±45	180±45	0.761
Mean LDL-C, mg/dL	102±29	100±36	0.198
Mean HDL-C, mg/dL	60±18	58±18	0.791
Median TGL, mg/dL	91 (74 – 121)	103 (94 – 117)	0.610
Median TGL/HDL ratio	1.6 (1.2 – 2.1)	1.9 (1.5 – 2.3)	0.139
IR	1 (10%)	1 (10%)	0.763
Mean Serum Albumin, g/L	43±3	43±3	0.616
Mean Uric Acid, mg/dL	5.4±1.0	5.7±1.1	0.312
Median Serum Iron, µg/dL	67 (59 – 85)	80 (66 – 123)	0.128
Questionnaires			
Mean FFQ score	4±2	4±1	1.000
Median IPAQ score	4290 (1680 – 9540)	4620 (3015 – 8445)	0.398
Metabolic syndrome			
WC>102 or >88, n (%)	4 (40%)	4 (40%)	0.675
TGL>150 or treatment mg/dl	7 (70%)	8 (80%)	0.500
DM or GLU>100 mg/dL	2 (20%)	3 (30%)	0.500
HTN or HTN treatment	9 (90%)	10 (100%)	0.500
HDL<40 or <50	1 (10%)	2 (20%)	0.500
MetS	2 (20%)	4 (40%)	0.314

BCM = body cell mass; BMI = body mass index; dBP = diastolic blood pressure; DM = diabetes mellitus; ECW = extra-cellular water; eGFR = estimated glomerular filtration rate; FM = fat mass; FFM = fat free mass; GLU = glucose; Hb = haemoglobin; HC = hips circumferences; HDL-C = high-density lipoprotein-cholesterol; HTN = hypertension; HTx = heart transplantation; ICW = intra-cellular water; IR = insulin resistance; LDL-C = low-density lipoprotein-cholesterol; LVEF = left ventricular ejection fraction; MetS = metabolic syndrome; MMF = mycophenolate; sBP = systolic blood pressure; SGOT = serum glutamic oxaloacetic transaminase; SGP = serum glutamyl pyruvic transaminase; sPAP = systolic pulmonary artery pressure; TBW = total body water; TGL= triglyceride; WBC = white blood cells; WC = waist circumferences.

5.3.6 Intervention group data comparison

The comparison of overall data from baseline to final timepoints of intervention group were analysed to assess any intra-group change during the study period.

Pharmacological therapy

As shown in Table 18, intervention group did not change the pharmacological therapy during all the study period. Both immunosuppressive treatment, hypertensive drugs and statins remained unchanged, with all participants taking statins for the three timepoints considered.

Table 18. Pharmacological therapy in intervention group during the study period.

Intervention group	Baseline (n. 11)	Intermediate (n.11)	p
Pharmacological therapy			
Cyclosporine	7 (64%)	7 (64%)	0.670
Everolimus	4 (36%)	4 (36%)	0.670
Tacrolimus	3 (27%)	3 (27%)	0.682
MMF	7 (64%)	7 (64%)	0.670
Corticosteroids	2 (18%)	2 (18%)	0.707
HTN treatment	9 (82%)	9 (82%)	0.707
Statins	11 (100%)	11 (100%)	
Median statin dose	20 (10 – 40)	20 (10 – 80)	0.317
	Intermediate (n.7)	**Final (n.7)**	**p**
Pharmacological therapy			
Cyclosporine	3 (43%)	3 (43%)	0.704
Everolimus	2 (29%)	2 (29%)	0.720
Tacrolimus	3 (43%)	3 (43%)	0.704
MMF	5 (71%)	5 (71%)	0.720
Corticosteroids	1 (14%)	1 (14%)	0.769
HTN treatment	6 (86%)	6 (86%)	0.769
Statins	7 (100%)	7 (100%)	-
Median statin dose	20 (10 – 80)	20 (20 – 40)	1.000
	Baseline (n.7)	**Final (n.7)**	**p**
Pharmacological therapy			
Cyclosporine	3 (43%)	3 (43%)	0.704
Everolimus	2 (29%)	2 (29%)	0.720
Tacrolimus	3 (43%)	3 (43%)	0.704
MMF	5 (71%)	5 (71%)	0.720
Corticosteroids	1 (14%)	1 (14%)	0.769
HTN treatment	6 (86%)	6 (86%)	0.769
Statins	7 (100%)	7 (100%)	-

Median statin dose	20 (10 – 400)	20 (20 – 40)	1.000

HTN= hypertension; MMF = mycophenolate.

Anthropometric, body composition and echocardiographic data

Anthropometric, body composition and echocardiographic data in intervention group during the study period are reported in Table 19. Mean weight and BMI remained comparable during the three timepoints, as well as hips circumference, with a tendency to a lower waist circumference at the intermediate timepoint (93.5±16.7 cm vs. 91.4±15.3 cm, p=0.152).

Body composition was, instead, different at every timepoint. Participants of the intervention group showed a significant lower mean FM% (23.1±7.1% vs. 18.4±7.7%, p=0.006), reaching the recommended range, already at meeting 2, after a median follow-up of 5.8 (4.7 – 6.3) months. The decrease pursued also to the final timepoint (19.0±8.5% vs. 14.8±10.1%, p=0.068), even if not significantly, obtaining a final mean FM% of 14.8±10.1% vs. baseline 23.2±6.3% (p=0.014). Subsequently, mean FFM% had a significant increase over the three timepoints (76.9±7.1% vs. 81.6±7.7%, p=0.006 for baseline and intermediate mean FFM%, respectively; 80.9±8.5% vs. 85.2±10.1%, p=0.068 for intermediate and final mean FFM%, respectively; 76.8±6.3% vs. 85.2±10.1%, p=0.014 for baseline and final mean FFM%, respectively). With the increase of mean FFM%, being muscle a hydrated tissue, also a consequent increase in TBW% was observed (58.6±5.4% vs. 61.5±5.3%, p=0.013 for baseline and intermediate mean TBW%, respectively; 61.5±5.3% vs. 64.0±6.3%, p=0.077 for intermediate and final mean TBW%, respectively; 58.4±4% vs. 64.0±6.3%, p=0.012 for baseline and final mean TBW%, respectively). Interestingly, between intermediate and final timepoints, also a significant increase in BCM% was observed (50.9±3.8% vs. 53.4±3.4%, p=0.031), as well as a decrease in ECW% (48.5±3.5% vs. 46.1±3.2%, p=0.030) and increase in ICW% (51.5±3.5% vs 53.9±3.2%, p=0.030).

Finally, both blood pressure and echocardiographic parameters remained comparable during the study period.

Table 19. Anthropometric, body composition and echocardiographic data in intervention group during the study period.

Intervention group	**Baseline (n.11)**	**Intermediate (n.11)**	**p**
Anthropometric and body composition data			
Mean weight, kg	77.6±18.6	77.3±18.0	0.698
Mean BMI, kg/m²	24.6±4.5	24.5±4.5	0.755
Mean WC, cm	93.5±16.7	91.4±15.3	0.152
Mean HC, cm	94.0±8.8	94.3±7.9	0.728
Mean WC/HC	0.99±0.13	0.96±0.12	0.214
Mean BCM%	52.0±4.7	51.8±3.3	0.790
Mean TBW%	58.6±5.4	61.5±5.3	**0.013**
Mean ECW%	47.4±4.4	47.6±3.1	0.761
Mean ICW%	52.6±4.4	52.4±3.1	0.761
Mean FM%	23.1±7.1	18.4±7.7	**0.006**
Mean FFM%	76.9±7.1	81.6±7.7	**0.006**
Echocardiographic data			
Median sPB, mmHg	130 (120 – 145)	130 (120 – 130)	0.610
Median dBP, mmHg	80 (78 – 85)	80 (75 – 80)	0.799
Mean LVEF, %	68±7	68±7	0.847
Mean sPAP	28±6	31±5	0.198
	Intermediate (n.7)	**Final (n.7)**	**p**
Anthropometric and body composition data			
Mean weight, kg	75.5±20.8	75.7±20.4	0.780
Mean BMI, kg/m²	24.1±5.1	24.1±5.1	0.801
Mean WC, cm	91.4±18.2	91.7±17.4	0.726
Mean HC, cm	92.3±8.1	92.1±9.3	0.865
Mean WC/HC	0.98±0.14	0.99±0.13	0.638
Mean BCM%	50.9±3.8	53.4±3.4	**0.031**
Mean TBW%	61.5±5.3	64.0±6.3	0.077
Mean ECW%	48.5±3.5	46.1±3.2	**0.030**
Mean ICW%	51.5±3.5	53.9±3.2	**0.030**
Mean FM%	19.0±8.5	14.8±10.1	0.068

Mean FFM%	80.9±8.5	85.2±10.1	0.068
Echocardiographic data			
Mean sPB, mmHg	130 (120 – 140)	130 (106 – 147)	0.715
Mean dBP, mmHg	80 (70 – 98)	80 (70 – 85)	0.705
Mean LVEF, %	72±6	71±5	0.772
Mean sPAP	31±4	29±6	0.509
	Baseline (n.7)	**Final (n.7)**	**p**
Anthropometric and body composition data			
Mean weight, kg	75.3±20.6	75.7±20.4	0.706
Mean BMI, kg/m^2	24.0±4.8	24.1±5.1	0.698
Mean WC, cm	93.0±17.9	91.7±17.4	0.243
Mean HC, cm	91.3±9.3	92.1±9.3	0.476
Mean WC/HC	1.01±0.15	0.99±0.13	0.224
Mean BCM%	51.1±4.6	53.4±3.4	0.171
Mean TBW%	58.4±4	64.0±6.3	**0.012**
Mean ECW%	48.2±4.3	46.1±3.2	0.194
Mean ICW%	51.8±4.3	53.9±3.2	0.194
Mean FM%	23.2±6.3	14.8±10.1	**0.014**
Mean FFM%	76.8±6.3	85.2±10.1	**0.014**
Echocardiographic data			
Mean sPB, mmHg	132±18	130±14	0.770
Mean dBP, mmHg	80±14	78±5	0.735
Mean LVEF, %	71±3	71±5	0.892
Mean sPAP	27±4	29±6	0.247

BCM = body cell mass; BMI = body mass index; dBP = diastolic blood pressure; ECW = extra-cellular water; rate; FM = fat mass; FFM = fat free mass; HC = hips circumferences; ICW = intra-cellular water; LVEF = left ventricular ejection fraction; sBP = systolic blood pressure; sPAP = systolic pulmonary artery pressure; TBW = total body water; WC = waist circumferences.

Blood parameters

Table 20 displays the blood parameters data in intervention group during the study period. All parameters remained comparable through the three timepoints. A tendency to a lower level of mean fasting GLU value was observed between baseline and meeting 2 (89±14 mg/dL vs. 84±10 mg/dL, p=0.078), however an increase occurred at final timepoint (86±13 mg/dL vs. 93±20 mg/dL, p=0.107). Similarly, also mean TGL value seemed to decrease at meeting 2 (95±43 mg/dL vs. 87±38 mg/dL, p=0.426), with, instead, an increase towards meeting 3 (81±44 mg/dL vs. 99±66 mg/dL, p=0.096). Cholesterol profile remained barely comparable, as well as renal and liver function.

Table 20. Blood parameters data in intervention group during the study period.

Intervention group	Baseline (n.11)	Intermediate (n.11)	p
Blood parameters			
Mean Hb, g/dL	14.3±1.5	14.2±1.8	0.859
Mean WBC, x10^3/µL	6.6±1.8	6.3±1.5	0.432
Mean GLU, mg/dL	89±14	84±10	0.078
Median Serum Creatinine, mg/dL	1.13 (1.05 – 1.43)	1.09 (1.07 – 1.26)	0.533
Median eGFR, mL/min/1.73mq	67 (61 – 77)	70 (64 – 77)	0.540
Median SGOT, UI/L	23 (18 – 31)	20 (18 – 28)	0.393
Median SGP, UI/L	18 (14 – 24)	19 (14 – 21)	0.373
Mean total Bilirubin, mg/dL	0.94±0.41	0.96±0.39	0.679
Mean Total Cholesterol, mg/dL	169±32	185±54	0.263
Median LDL-C, mg/dL	92 (70 – 104)	102 (72 – 128)	0.285
Mean HDL-C, mg/dL	60±15	60±12	1.000
Mean TGL, mg/dL	95±43	87±38	0.426
Mean TGL/HDL ratio	1.74±1.06	1.54±0.79	0.401
IR	2 (18%)	1 (9%)	0.500
Mean Serum Albumin, g/L	45±3	46±3	0.593
Mean Uric Acid, mg/dL	5.6±0.9	5.7±0.9	0.803
Mean Serum Iron, µg/dL	84±15	79±18	0.469
	Intermediate (n.7)	**Final (n.7)**	**p**
Blood parameters			
Mean Hb, g/dL	13.7±2.0	14.2±1.8	0.123
Mean WBC, x10^3/µL	5.90±1.57	6.36±2.44	0.403
Mean GLU, mg/dL	86±13	93±20	0.107
Median Serum Creatinine, mg/dL	1.09 (1.05 – 1.19)	1.13 (1.01 – 1.23)	0.463
Mean eGFR, mL/min/1.73mq	70 (67 – 75)	71 (66 – 79)	1.000
Mean SGOT, UI/L	20 (19 – 25)	21 (20 – 26)	0.916
Median SGP, UI/L	19 (17 – 21)	21 (17 – 25)	0.916

Mean total Bilirubin, mg/dL	0.79±0.27	0.78±0.30	0.861
Mean Total Cholesterol, mg/dL	169±38	184±44	0.218
Mean LDL-C, mg/dL	102 (66 – 104)	97 (80 – 112)	0.500
Mean HDL-C, mg/dL	63±13	66±15	0.190
Mean TGL, mg/dL	81±44	99±66	0.096
Mean TGL/HDL ratio	1.40±0.90	1.70±1.44	0.200
IR	1 (14%)	1 (14%)	0.769
Mean Serum Albumin, g/L	45±3	46±6	0.637
Mean Uric Acid, mg/dL	5.4±1.0	5.8±1.5	0.625
Mean Serum Iron, µg/dL	71±12	76±21	0.605
	Baseline (n.7)	**Final (n.7)**	**p**
Blood parameters			
Mean Hb, g/dL	13.9±1.8	14.2±1.8	0.422
Mean WBC, x10^3/µL	6.28±2.15	6.36±2.44	0.780
Mean GLU, mg/dL	94±16	93±20	0.735
Median Serum Creatinine, mg/dL	1.19 (1.05 – 1.25)	1.13 (1.01 – 1.23)	0.674
Mean eGFR, mL/min/1.73mq	65±21	64±21	0.815
Mean SGOT, UI/L	23±7	22±5	0.893
Median SGP, UI/L	19	21 (17 – 25)	0.733
Mean total Bilirubin, mg/dL	0.79±0.36	0.78±0.30	0.888
Mean Total Cholesterol, mg/dL	169±39	184±44	0.189
Mean LDL-C, mg/dL	86±23	99±33	0.136
Mean HDL-C, mg/dL	66±15	66±15	1.000
Median TGL, mg/dL	83±42	99±66	0.368
Mean TGL/HDL ratio	1.32±0.66	1.70±1.44	0.360
IR	2 (15%)	1 (14%)	0.730
Mean Serum Albumin, g/L	46±4	46±6	0.723
Mean Uric Acid, mg/dL	5.2±0.9	5.8±1.5	0.372
Mean Serum Iron, µg/dL	84±20	76±21	0.302

eGFR = estimated glomerular filtration rate; GLU = glucose; Hb = haemoglobin; HDL-C = high-density lipoprotein-cholesterol; IR = insulin resistance; LDL-C = low-density lipoprotein-cholesterol; SGOT = serum glutamic oxaloacetic transaminase; SGP = serum glutamyl pyruvic transaminase; TGL= triglyceride.

Questionnaires

The results of FFQ and IPAQ questionnaires in intervention group during the study period are shown in Table 21. While IPAQ score seemed to increase through the three timepoints, without reaching a significant difference, results from FFQ reported a progressive significant increase in the adherence to the MedDiet. Although only a tendency at the meeting 2 (mean FFQ score 4±2 vs. 5±2, p=0.117), adherence to MedDiet became significantly greater at the final meeting (median FFQ score 6, range 5 – 6 vs. 7, range 7 – 8, p=0.026), with an overall increase started from a baseline median score of 4 (4 – 5) to a final median score of 7 (7 – 8) (p=0.017). Table 22 displays the FFQ items comparison utilised to calculate the MedDiet score, between baseline and final timepoints. The main improvements were observed in olive oil (14% vs. 100%, p=0.002 for baseline and final timepoints, respectively), fish (29% vs. 41%, p=0.143 for baseline and final timepoints, respectively) and nuts (43% vs. 86%, p=0.133 for baseline and final timepoints, respectively) consumption.

Table 21. FFQ and IPAQ results in intervention group during the study period.

Intervention group	**Baseline (n.11)**	**Intermediate (n.11)**	**p**
Mean FFQ score	4±2	5±2	0.117
Median IPAQ score	2760 (1526 – 5048)	4845 (3038 – 5373)	0.655
	Intermediate (n.7)	**Final (n.7)**	**p**
Median FFQ score	6	7 (7 – 8)	**0.026**
Median IPAQ score	4845 (3038 – 5373)	8295 (1883 – 14865)	0.686
	Baseline (n.7)	**Final (n.7)**	**p**
Median FFQ score	4 (4 – 5)	7 (7 – 8)	**0.017**
Median IPAQ score	2760 (2520 – 8220)	8295 (1883 – 14865)	0.686

Table 22. FFQ items comparison between baseline and final timepoints in intervention group.

Intervention group	Baseline = 7	Intermediate = 7	p
Wholegrain products ≥1/day, n (%)	4 (57)	4 (57)	0.704
Vegetables ≥2/day, n (%)	5 (71)	7 (100)	0.231
Fruits ≥2/day, n (%)	3 (43)	5 (71)	0.296
Olive oil ≥3/day, n (%)	1 (14)	7 (100)	0.002
Wine 1-27day for men; ≤1/day for women, n (%)	1 (14)	3 (43)	0.280
Read meat ≤3/week, n (%)	7 (100)	7 (100)	
Fish ≥2/week, n (%)	2 (29)	5 (41)	0.143
Nuts ≥2/week, n (%)	3 (43)	6 (86)	0.133
Legumes ≥2/week, n (%)	2 (29)	4 (57)	0.296

5.3.7 Intervention group food diaries analysis

The food diaries that participants of intervention group filled during the study period, at baseline, meeting 2 and meeting 3, were analysed as mentioned in Methods. The results were divided in macronutrients and micronutrients analysis.

Macronutrients

Comparison of macronutrients consumption are reported in Table 23. The main result is represented by the significant decrease of the mean percentage of energy intake (%E) from saturated fatty acids (SFA): while only a tendency between baseline and meeting 2 (12.6±2.4% vs. 11.0±3.0%, p=0.134), the decrease became significant between meeting 2 and 3 (11.3%, range 10.7 – 14.2% vs. 10.3%, range 8.5 – 10.7%, p=0.018), with an overall decrease starting from 13.0±2.1% at baseline to 9.6±1.5% at final timepoint (p=0.001), reaching the recommended Reference Intake (RI) (<10% of total energy intake)(96). Even if not significative, an increase in %E from carbohydrates was detected both between baseline and meeting 2 (44.4±5.3% vs. 45.8±7.2%, p=0.295) and between meeting 2 and 3 (42.2±7.1% vs. 45.7±5.7%, p=0.099), reaching an overall increase of 41.7±4.0% vs. 45.7±5.7% at baseline vs. final timepoint respectively (p=0.081), in line with the RI(96). The mean proteins consumption had a mild decrease, especially between intermediate and final timepoints (65.7±8.3g vs. 58.0±6.6g, p=0.094). Similar remark for mean %E from fat, with a trend to decrease between meeting 2 and 3 (40.0±6.0% vs. 35.4±4.5%, p=0.094), and an overall decrease of 38.0±3.5% vs. 35.4±4.5% at baseline vs. final timepoint respectively (p=0.284), almost reaching the RI (<35% of total energy intake)(96). Instead, even if the reduction was not statistically significant, mean %E of sugars met the RI of <15% in the final timepoint (15.4±3.0% vs. 14.6±2.0%, p=0.541 for baseline and final timepoints, respectively). On the other hand, a negative trend was detected in omega-3 EPA and DHA between intermediate and final timepoints (median EPA 0.11g, range 0.04 – 0.45g vs. 0.03g, range 0.01 – 0.06g, p=0.091; mean DHA 0.50±0.63g vs. 0.08±0.08g, p=0.150), with a consequent median sum EPA + DHA of 0.34g, range 0.08 – 1.22g vs. 0.07g, range 0.04 – 0.17g (p=0.091) between intermediate and final timepoints and of 0.09g, range 0.04 – 0.52g vs. 0.07g, range 0.04 – 0.17g, p=0.612 between baseline and final timepoints, still far from the 0.25g recommended by LARN(96). A trend to a lower mean cholesterol level was observed between baseline and final meeting (219±82g vs. 171±59g, p=0.082), even if it resulted in line with recommendations at each timepoint (<300 mg/day)(96). Finally, also a trend to a higher mean fibres level was observed between baseline and final meeting (15.2±4.8g vs. 18.3±3.0g, p=0.100), although not reaching yet the ≥25g/day recommended by LARN (96).

Table 23. Food diaries analysis: comparison of macronutrients consumption in intervention group during the study period.

Intervention group	**Baseline (n.11)**	**Intermediate (n. 11)**	**p**	**LARN Reference Value**
Mean Energy, Kcal	1722±376	1671±215	0.535	
Mean %E Carbohydrate	44.4±5.3	45.8±7.2	0.295	45 – 60%
Mean %E Sugars	15.5±3.6	15.4±4.2	0.993	<15%
Mean Fibres, g	16.2±6.4	17.6±5.6	0.322	≥25g
Mean %E Proteins	15.1±2.26	14.8±2.1	0.412	
Mean Proteins, g	63.9±12.0	61.5±9.7	0.445	
Mean %E Fat	36.5±4.2	36.7±6.6	0.931	20 – 35%
Mean %E SFA	12.6±2.4	11.0±3.0	0.134	<10%
Mean %E MUFA	15.5±3.0	16.5±2.3	0.320	10-15%
Mean Oleic acid (18:01), g	28.1±9.4	29.2±5.7	0.664	
Median %E PUFA	3.67 (3.24 – 4.22)	3.95 (3.41 – 4.52)	0.530	5 – 10%
Median Linoleic acid (18:02), g	4.98 (4.53 – 6.53)	5.48 (4.86 – 6.47)	0.583	
Median α-linoleic acid (18:03), g	1.09 (0.70 – 1.23)	0.83 (0.79 – 1.13)	0.754	
Median Arachidonic acid (20:04), g	0.18 (0.11 – 0.30)	0.12 (0.08 – 0.14)	0.015	
Median EPA (20:05), g	0.05 (0.04 – 0.10)	0.05 (0.03 – 0.27)	0.583	
Median DHA (22:06), g	0.07 (0.04 – 0.15)	0.04 (0.02 – 0.43)	0.722	
Median EPA + DHA, g	0.12 (0.08 – 0.25)	0.08 (0.06 – 0.72)	1.000	0.25 g
Mean Cholesterol, g	234±72	184±88	0.169	<300 mg
	Intermediate (n.7)	**Final (n.7)**	**p**	**LARN Reference Value**
Mean Energy, Kcal	1713±219	1637±254	0.452	
Mean %E Carbohydrates	42.2±7.1	45.7±5.7	0.099	45 – 60%
Median %E Sugars	17.3 (15.6 – 18.3)	15.0 (13.0 – 15.7)	0.176	<15%
Mean Fibres, g	16.6±4.3	18.3±3.0	0.486	≥25g
Mean %E Proteins	15.5±2.3	14.3±1.5	0.273	

Mean Proteins, g	65.7±8.3	58.0±6.6	0.094	
Mean %E Fat	40.0±6.0	35.4±4.5	0.094	20 – 35%
Median %E SFA	11.3 (10.7 – 14.2)	10.3 (8.5 – 10.7)	0.018	<10%
Mean %E MUFA	16.8±2.0	16.5±4.0	0.850	10-15%
Median Oleic acid (18:01), g	28.6 (27.7 – 28.7)	29.5 (25.6 – 30.2)	0.310	
Median %E PUFA	4.29 (3.87 – 5.10)	3.85 (3.74 – 4.58)	0.866	5 – 10%
Median Linoleic acid (18:02), g	6.41 (5.34 – 7.18)	6.61 (5.61 – 7.47)	1.000	
Median α-linoleic acid (18:03), g	1.03 (0.83 – 1.50)	1.07 (0.73 – 1.29)	0.310	
Median Arachidonic acid (20:04), g	0.10 (0.07 – 0.13)	0.09 (0.09 – 0.12)	0.735	
Median EPA (20:05), g	0.11 (0.04 – 0.45)	0.03 (0.01 – 0.06)	0.091	
Mean DHA (22:06), g	0.50±0.63	0.08±0.08	0.150	
Median EPA + DHA, g	0.34 (0.08 – 1.22)	0.07 (0.04 – 0.17)	0.091	0.25 g
Mean Cholesterol, g	200±78	171±59	0.468	<300 mg
	Baseline (n.7)	**Final (n.7)**	**p**	**LARN Reference Value**
Mean Energy, Kcal	1745±465	1637±254	0.379	
Mean %E Carbohydrates	41.7±4.0	45.7±5.7	0.081	45 – 60%
Mean %E Sugars	15.4±3.0	14.6±2.0	0.541	<15%
Mean Fibres, g	15.2±4.8	18.3±3.0	0.100	≥25g
Mean %E Proteins	15.7±2.6	14.3±1.5	0.169	
Mean Proteins, g	67.1±14.4	58.0±6.6	0.129	
Mean %E Fat	38.0±3.5	35.4±4.5	0.284	20 – 35%
Mean %E SFA	13.0±2.1	9.6±1.5	0.001	<10%
Mean %E MUFA	16.6±2.3	16.5±4.0	0.963	10-15%
Mean Oleic acid (18:01), g	30.1±9.7	28.3±4.7	0.639	
Median %E PUFA	4.03 (3.56 – 5.07)	3.85 (3.74 – 4.58)	0.499	5 – 10%
Median Linoleic acid (18:02), g	6.25 (4.54 – 7.69)	6.61 (5.61 – 7.47)	0.866	
Mean α-linoleic acid (18:03), g	1.22±0.30	1.13±0.58	0.758	

Mean Arachidonic acid (20:04), g	0.26±0.23	0.11±0.07	0.167	
Median EPA (20:05), g	0.04 (0.01 – 0.22)	0.03 (0.01 – 0.06)	0.497	
Median DHA (22:06), g	0.07 (0.02 – 0.30)	0.06 (0.04 – 0.11)	0.735	
Median EPA + DHA, g	0.09 (0.04 – 0.52)	0.07 (0.04 – 0.17)	0.612	0.25 g
Mean Cholesterol, g	219±82	171±59	0.082	<300 mg

%E = % Energy; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids; SFA = saturated fatty acids.

* RI for men/RI for women for adults aged 30-59 years

Micronutrients

Table 24 displays the analysis of micronutrients between the three timepoints.

First of all, a significant decrease in mean Na intake was observed between meeting 2 and 3 (2138±359mg vs. 1822±417mg, p=0.045), although the statistical significance was lost comparing baseline to meeting 3 (2174±840mg vs. 1822±417mg, p=0.238). Anyway, the final mean Na value was closer to the recommended 1500mg/day of RI (96).

For Mg intake, instead, while the baseline vs. intermediate values were under the range (153±70mg vs. 160±70mg, p=0.619), both at the intermediate vs. final (193±64mg vs. 178±52mg, p=0.294) and baseline vs. final (172±83mg vs. 178±52mg, p=0.800) comparisons the values resulted reaching the recommended 170mg/day from LARN (96).

Mean Iron intake resulted in a slight increase at all the timepoints (8.74±2.84mg vs. 9.40±1.89mg, p=0.451, for baseline and intermediate timepoints, respectively; 9.47±1.98mg vs. 10.13±2.89mg, p=0.423, for intermediate and final timepoints; 9.49±2.46mg vs. 10.13±2.89mg, p=0.513, for baseline and final timepoints), with all values fitting the recommended range (7mg for men/ 10mg for women for adults aged 30-59 years) (96).

Also mean Se intake showed a mild increase between baseline and final timepoints (28.1±12.5µg vs. 32.6±16.1µg, p=0.513), although without yet reaching the reference value (45µg/day) (96).

A rising trend of mean α-tocopherol equivalent was detected at all comparisons (9.61±2.12mg vs. 10.53±1.48mg, p=0.081, for baseline and intermediate timepoints, respectively; 10.56±0.92mg vs. 11.25±0.65mg, p=0.092, for intermediate and final timepoints; 9.64±1.54mg vs. 11.25±0.65mg, p=0.062, for baseline and final timepoints), with a final value very close to the RI (13mg for men/ 12mg for women for adults aged 30-59 years) (96). VitC and Retinol-equivalent, instead, had in-target mean values at all the timepoints.

As regards the B groups Vitamins, both Vit2, 3, 6 and 12 resulted satisfying the recommended daily intake at all three timepoints.

Table 24. Food diaries analysis: comparison of micronutrients consumption in intervention group during the study period.

Intervention group	Baseline (n.11)	Intermediate (n.11)	p	LARN Reference Value
Median Na, mg	2029 (1765 – 2263)	2092 (1713 – 2240)	0.583	1500
Mean K, mg	2240±513	2408±486	0.239	3900
Mean Ca, mg	603±305	565±182	0.443	800
Mean Mg, mg	153±70	160±70	0.619	170
Mean P, mg	954±282	918±204	0.603	580
Mean Iron, mg	8.74±2.84	9.40±1.89	0.451	7/10*
Median Cu, mg	0.47 (0.39 – 0.67)	0.49 (0.48 - 0.80)	0.505	0.7
Median Zinc, mg	7.71 (6.44 – 8.06)	7.47 (6.92– 8.37)	0.875	10/8*
Median Se, µg	23.19 (16.38 – 29.51)	22.21 (16.39 – 30.55)	0.937	45
Median Chlor, mg	1404 (1131 – 2212)	1411 (1372 – 1524)	0.239	2300
Mean Mn, mg	0.62±0.40	0.95±0.60	0.013	2.7/2.3*
Mean Iodine, µg	64.7±37.4	82.7±42.2	0.104	150
Mean Retinol-equivalent, µg	767±318	767±407	0.992	500/400*
Mean VitC, mg	122±61	126±44	0.891	75/60*
Median VitD, µg	1.92 (1.08 – 2.18)	1.67 (0.79 – 4.39)	0.754	10
Mean α-tocopherol equivalent, mg	9.61±2.12	10.53±1.48	0.081	13/12*
Median VitK, µg	3.49 (1.59 – 10.42)	6.79 (3.65 – 11.48)	0.534	140
Median VitB1, mg	0.78 (0.73 – 0.94)	0.87 (0.53 – 1.61)	0.583	1/0.9*
Mean VitB2, mg	1.36±0.31	1.41±0.40	0.688	1.3/1.1*
Median VitB6, mg	1.35 (1.23 – 1.56)	1.63 (1.35 – 1.97)	0.182	1.1
Mean VitB12, µg	3.24±1.25	3.32±1.73	0.884	2.0
Mean Niacin, mg	15.6±5.3	16.9±5.1	0.480	14
Median Biotin, µg	16.37 (11.30 – 20.43)	17.47 (16.14 – 19.70)	0.530	30
Median Pantothenic acid, mg	2.07 (1.68 – 2.32)	2.38 (1.88 – 3.06)	0.347	5.0
Mean Folate, µg	243±71	266±67	0.472	320

	Intermediate (n.7)	Final (n.7)	p	LARN Reference Value
Mean Na, mg	2138±359	1822±417	0.045	1500
Mean K, mg	2557±374	2523±356	0.821	3900
Mean Ca, mg	640±163	423±164	0.029	800
Mean Mg, mg	193±64	178±52	0.294	170
Mean P, mg	983±169	862±155	0.043	580
Mean Iron, mg	9.47±1.98	10.13±2.89	0.423	7/10*
Mean Cu, mg	0.66±0.28	0.56±0.15	0.137	0.7
Mean Zinc, mg	7.86±1.06	7.27±1.54	0.160	10/8*
Mean Se, µg	39.9±27.8	32.6±16.1	0.272	45
Median Chlor, mg	1442 (1409 – 1757)	1449 (1178 – 1686)	0.091	2300
Mean Mn, mg	1.08±0.70	0.86±0.30	0.427	2.7/2.3*
Mean Iodine, µg	104±38	70.3±40.4	0.010	150
Mean Retinol-equivalent, µg	759±366	943±747	0.629	500/400*
Mean VitC, mg	141±45	131±40	0.729	75/60*
Mean VitD, µg	4.26±4.28	1.79±0.97	0.155	10
Mean α-tocopherol equivalent, mg	10.56±0.92	11.25±0.65	0.092	13/12*
Mean VitK, µg	5.65±4.06	8.48±5.09	0.295	140
Median VitB1, mg	0.81 (0.78 – 1.01)	0.88 (0.72 – 0.97)	0.612	1/0.9*
Mean VitB2, mg	1.41±0.17	1.28±0.35	0.327	1.3/1.1*
Mean VitB6, mg	1.63±0.40	1.62±0.18	0.947	1.1
Mean VitB12, µg	3.65±1.66	2.71±1.17	0.115	2.0
Median Niacin, mg	15.1 (14.5 – 19.4)	13.9 (16.5 – 20.0)	0.237	14
Mean Biotin, µg	19.2±3.7	16.4±4.0	0.083	30
Mean Pantothenic acid, mg	2.78±1.12	2.26±0.63	0.113	5.0
Mean Folate, µg	270±40	242±44	0.180	320

	Baseline (n.7)	Final (n.7)	p	LARN Reference Value
Mean Na, mg	2174±840	1822±417	0.238	1500
Mean K, mg	2357±584	2523±356	0.219	3900
Mean Ca, mg	738±344	423±164	0.022	800
Mean Mg, mg	172±83	178±52	0.800	170
Mean P, mg	1072±303	862±155	0.071	580
Mean Iron, mg	9.49±2.46	10.13±2.89	0.494	7/10*
Median Cu, mg	0.52 (0.46 – 0.67)	0.54 (0.46 – 0.63)	0.735	0.7
Median Zinc, mg	7.65 (6.41 – 7.83)	7.20 (6.67 – 7.55)	0.398	10/8*
Mean Se, µg	28.1±12.5	32.6±16.1	0.513	45
Mean Chlor, mg	1991±983	1418±366	0.074	2300
Mean Mn, mg	0.62±0.36	0.86±0.30	0.206	2.7/2.3*
Mean Iodine, µg	75.6±45.6	70.3±40.4	0.704	150
Median Retinol-equivalent, µg	691 (589 – 833)	698 (558 – 935)	1.000	500/400*
Mean VitC, mg	125±70	131±40	0.740	75/60*
Median VitD, µg	1.80 (1.06 – 2.06)	1.93 (1.27 – 2.44)	0.310	10
Mean α-tocopherol equivalent, mg	9.64±1.54	11.25±0.65	0.062	13/12*
Mean VitK, µg	3.63±3.39	8.48±5.09	0.020	140
Mean VitB1, mg	0.95±0.28	0.86±0.17	0.480	1/0.9*
Mean VitB2, mg	1.46±0.35	1.28±0.35	0.138	1.3/1.1*
Mean VitB6, mg	1.56±0.46	1.62±0.18	0.700	1.1
Mean VitB12, µg	3.45±1.57	2.71±1.17	0.076	2.0
Median Niacin, mg	16.0 (13.0 – 20.7)	13.9 (16.5 – 20.0)	0.735	14
Mean Biotin, µg	17.6±8.6	16.4±4.0	0.709	30
Mean Pantothenic acid, mg	2.54±1.23	2.26±0.63	0.499	5.0
Mean Folate, µg	248±72	242±44	0.876	320

Ca = calcium; Chlor = chloride; Cu = copper; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; P = phosphorus; Se = selenium; Vit = vitamin.

* RI for men/RI for women for adults aged 30-59 years

5.3.8 Discussion

The main findings of this study were: 1) Mediterranean diet adherence significantly increased in the intervention group during the study period, both comparing intervention and control group and within the intervention group over the three timepoints; 2) Accordingly, body composition of intervention group significantly changed over the study period, with a significant decrease in fat mass % and a subsequent increase in fat free mass % and body cell mass %; 3) As a confirmation, dietary habits of intervention group enhanced over the study period, with an improvement in macronutrients balance, a significant decrease of energy from saturated fatty acids and soluble sugars, and a positive trend to micronutrients intake.

Firstly, to our knowledge, only three research papers have assessed the diet in HTx patients and evaluated the advantages, in terms of prevention, of a structured dietary intervention in this population (Table 2). Of these papers, one is focused on the AHA diet (97), while the other two reported the results of a MedDiet-dietary pattern intervention (60, 61). Among all dietary patterns, MedDiet has proved, in fact, to be the most effective in reducing the cardiovascular risk factors (65, 66), as well as in reversing and limiting the Metabolic syndrome development in general adult population (68, 69). However, none of these studies included a control group in the analysis. In our study, intervention group received, at baseline, personalised and structured nutritional advices provided by a trained nutritionist and based on the MedDiet recommendations. Successively, they were monitored over other two meeting, reaching a total of 10-12 months of follow-up. Control group, instead, received general indications at baseline from the Guidelines for a healthy nutrition (95), as shown in Appendix C, without other scheduled meetings until the final timepoint (98).

As a matter of fact, the adherence to MedDiet, similar at baseline between the two groups (mean score 4±2 for both), resulted in a progressive increase in intervention group, until a significant improvement at the end of the study period ($p=0.017$). The final median score on intervention group reached 7 points (range 4 - 8), beyond the cut-off of 6 points for the "good adherence" evaluation. Similarly, also Entwistle et al. obtained a significant improvement in the adherence to MedDiet after 52 weeks of dietary intervention, in their heart or lung transplanted patients population (59). Other data in solid organ transplanted patients are lacking. This result seems to demonstrate that a structured and monitored dietary intervention, exerted by a competent figure like a nutritionist, rather than general nutritional indications, is feasible and highly effective in improving the dietary habits of HTx patients.

Accordingly, body composition of intervention group patients significantly changed over the study period. A huge reduction in FM% was observed already at the second meeting ($p=0.006$), starting

with a baseline mean FM% of 23.1±7.1% and reaching a mean 18.4±7.7%, confirmed also at the final meeting (p=0.014). Therefore, while at baseline patients exceeded the recommended range of 15-23% for a good physical form, they achieved an adequate FM% at the end of the dietary intervention. Subsequently, FFM% had an important surge, passing from an out-of-range mean FFM% of 76.9±7.1% at baseline, to a final 85.2±10.1%. Considering that FFM includes the skeleton, body fluids, skin, organs and muscles, and considering that a rise in BCM% was also observed, we can speculate that the increase concerned the muscle component. For this reason, taking into account that muscle mass is highly hydrated and, thus, weights more than fat mass, the absence of weight-loss observed should not surprise us. Rather, we pointed out an interesting body re-composition, with an important loss in FM%. Entwistle et al. also reported a non-significant change in both weight and BMI, in their heart or lung transplanted patients after 52 weeks of dietary intervention (10, 60). Their explanation was a probable change in muscle density that they did not evaluate with body composition analysis, while this study could potentially confirm this assumption, as described above.

Together with the enhancement in the dietary habits, also IPAQ questionnaire seemed to outline an improvement in the physical activity of these patients, although not significantly, confirming even more the observed changes in body composition.

This result encompasses two main important achievement:

1) Fat mass, especially visceral adipose tissue, is strictly associated with MetS and cardiovascular disease (31, 60), thus its reduction may possibly translate in a reduced cardiovascular risk and a regression of MetS in these patients;

2) Low muscle mass has been proved to be an independent predictor of mortality and major morbidity after HTx, as well as in non-cardiac solid-organ transplants(99), hence maintaining an adequate muscles compartment could represent a protective factor.

Similarly, also the control group showed a reduction in FM% and increase in FFM% during the study period, even if the difference did not reach the statistical significance. As a matter of fact, when comparing the two groups, no difference was found at the intermediate timepoint. A possible explanation could be linked to the general nutritional advices given to these patients at baseline by the nutritionist: even if not structured and personalized as in intervention group, these healthy diet indications could have had an impact on dietary habits of control group patients. Anyway, the structured dietary intervention led to more evident changes in body composition, letting us speculating that a monitored education could be more beneficial.

Another study by Fernandez de la Puebla and colleagues observed a similar change in body composition of their 34 hypercholesterolemic adult males enrolled, after only 28 days of MedDiet

intervention. In this case, a decrease in fat mass was noted when changing from a saturated fat diet (mean fat mass 23.3±6.3 kg) to MedDiet (mean fat mass 20.8±7.2 kg, $p<0.05$)(100). In the PREDIMED study by Alvarez-Perez et al., though, after 1 year of MedDiet intervention on 351 Canarian subjects aged 55 to 80 years, with type 2 diabetes or ≥3 cardiovascular risk factors, they did not find any relevant body composition change associated with MedDiet. On the contrary, they observed significant within-group reductions in all anthropometric variables, including weight, BMI and WC (101). In our research, we did not find significant changes in the anthropometric variables considered, even if we did observe a reduction in WC over the study period, within both the intervention and control group.

Waist circumferences, rather than BMI, is reported to be one of the most important factors for obesity-related health risk (102). In this research, we observed a mean WC reduction of -1.3±2.6cm in patients of intervention group between baseline and final timepoints. The result did not reach the statistical significance, probably due to the small patients sample, but this decrease is greater than the -0.9cm average revealed in a MedDiet meta-analysis (103), and -0.55cm in the MedDiet+EVOO PREDIMED trial findings (68).

In the comparison between the two groups, both systolic and diastolic blood pressure resulted significantly lower in intervention group at the final timepoint. However, there was not any evident change in these parameters within the single groups. Of interest, a great reduction in Na consumption was observed in intervention group, over the study period, from the analysis of the food diaries. The median Na value at baseline was 2.0g, +0.5g over the LARN Reference Value (96), and decreased to a value of 1.8g at the final meeting. This result is important because a high dietary intake of Na is associated with high risk of elevated blood pressure and cardiovascular disease (104), but, above all, Braith et al. demonstrated that blood pressure in HTx recipients is salt sensitive (105). Therefore, a lower intake of Na could have led to the outcome observed in the intervention group.

Similarly, renal function, expressed in the study with the eGFR, appeared significantly better in the intervention group, compared to the control group, at the final comparison. Obviously, the higher age of the control group may have contributed to this result, since there is a well-recognised correlation with kidney function decline (106).

Compelling evidence from basic science, population studies, and clinical trials implicates soluble sugars as playing a major role in the development of HTN too, and may contribute to overall cardiovascular risk through a variety of mechanisms (107). The World Health Organization (54) has issued guidelines recommending that added sugars should constitute no more than 5% of overall calorie consumption (108). A less restrictive limit of <15% is instead indicated in the LARN

recommendations (96). Patients of our study started with an out of range mean %E sugars of 15.4±3.0% at baseline, to a final in-range mean %E sugar of 14.6±2.0%. In summary, MedDiet is not strictly a low-salt diet, but it is characterised by the consumption of mainly unprocessed foods, which could explain the lowering of both Na and sugars.

For what concern lipid profile, no differences were observed, after the study period, between intervention and control group, which, anyway, remained in the target clinical range. However, mean TGL level tended to be lower in intervention group (p=0.153). Entwistle and colleagues, instead, did find a significant reduction in TGL level, at the end of their 52 weeks of MedDiet intervention in 20 heart or lung transplanted patients, together with a decrease in total cholesterol and LDL-cholesterol (10), in line with the other French study on HTx recipients (61). One reason why we did not find this same outcome could lie in the smaller size of our patients sample.

The relationship between high level of TGL and various atherogenic lipoproteins increases the residual risk of CVD even when LDL measurements are brought within target range using statin therapy. Hypertriglyceridemia presents a significant risk factor for future cardiovascular events and is frequently encountered in type 2 DM and MetS patients (109). In their analysis of dietary fat and carbohydrate intake in type 2 DM patients, Vitale et al. demonstrated that it is the quality of carbohydrate, subsequent glycaemic load and fibre component that are responsible for influencing serum triglycerides (110). Indeed, patients from intervention group did increase their fibre consumption over the study period and this result, even if did not reach the statistical significance, could represent a contributing factor to fats and sugars decreased consumption, increasing the quality of the diet. The fibre consumption switched, in fact, from a baseline mean value of 15.2±4.8g to a final 18.3±3.0g (p=0.100). This last value did not reach yet the recommended ≥25g from LARN (96), but the trend appeared rising.

The decreasing TGL trend was correlated with a lower TGL/HDL ratio in intervention group compared to control group. This ratio provides a robust and predictive capacity for determining CVD and all-cause mortality risk in adult population (111). A ratio of <2 constitutes the desired target range in at risk populations, which suggests our baseline values, all of them on average <2, were clinically well controlled. Moreover, both Biadi et al (49) and Raichlin and colleagues (50) reported that a TG/HDL ratio of >3, used as an index of IR, in combination with a CRP reading >3ml/L were significantly associated with CAV incidence.

A last result in the blood parameters worth to mention is a trend of reduction in fasting GLU observed in the intervention group, compared to the control group. This reduction is also evident within the intervention group, from baseline to intermediate timepoint (p=0.078). Since dysglycemia increases

significantly in the long-term post HTx, as well as DM prevalence (1, 15), a good control of fasting GLU is essential. In general populations, a fasting GLU reading of 100mg/dL or greater is accepted as the threshold for impaired glucose tolerance (112), while in their large single-centre study, Rosettenstein et al. identified new onset diabetes after transplantation (NODAT) in 73% of kidney transplant recipients with a fasting GLU reading of ≥100mg/dL(113). Considering that Tacrolimus increases the risk of diabetes mellitus, the change in dietary habits proved in intervention group may have played a major role in GLU reduction, especially as regard improvement in dietary fibre. Fibres present during the meal, in fact, delay the nutrients adsorption during the post-prandial phase, with a consequent delay of the adsorption of sugars and, thus, the glycaemic peak (83, 84). However, also high fat diets increase the risk of hyperglycaemia of 1.3–1.6 times, particularly diets rich in SFA (114). Intriguingly, the dietary changes observed from the food diaries analysis pointed out an overall balance of macronutrients intake, including fat intake. The macronutrients distribution goal from the LARN recommendations is a carbohydrates percentage ranged 45 – 60%, a fat percentage ranged 20 – 35%, and about 15% for proteins (96). Our patients passed from a mean of 42%, 38% and 16% for carbohydrates, fat and proteins intake respectively at baseline, showing a modest misbalance, to a more balanced mean of 46%, 35% and 14% at the end of the study. An important result which, however, could be further improved by 1) increasing the consumption of wholegrain products, fruits and vegetables to reach a more beneficial carbohydrates percentage of 50-55%, and improving fibre intake to achieve the recommended value; 2) decreasing even more the fat intake to reach a percentage of 25-30%, preferring the MUFA and PUFA rather than SFA; 3) keeping a moderate consumption of proteins, to preserve the renal function. Considering the positive trend observed, all this achievements can be reached keeping following the dietary indications received, thus a constant and continuative nutritional monitoring may be preferable and beneficial.

The important point to highlight here is that all the indications aimed to educate the patients towards a healthier diet, rather than drastically reduce or restrict specific foods. This consideration is particularly fitting as regard fat intake. An overall limitation on total fat inevitably lowers intake of unsaturated fats, which, among them, omega-3 PUFA are particularly healthful (31, 87), while a preferable target would be the limitation of SFA. There are controversial results, in literature, as regard the association between SFA intake, all-cause mortality and CVD risk. In their meta-analysis of 21 prospective cohort studies, involving 347,747 subjects, Siri-Tarino and colleagues showed an absence of evidence for concluding that dietary SFA are effectively associated with an increased risk of CVD (115). However, a meta-analysis of randomised controlled trials, with about 59,000 participants, found that reducing dietary SFA intake, the risk of CVD events decreased by 17%, and

the replacement of SFA with PUFA was associated with a statistically significant 27% reduction in CVD events (116). An even bigger and more recent meta-analysis by Mazidi et al, involving 29 prospective cohort studies with a total of 1,148,117 participants, stated that SFA intake was associated with all-cause, coronary heart disease (CHD), stroke and type 2 DM mortality (117). All these evidences suggest that reducing SFA and replacing them with PUFA, may reduce risks of CVD and CHD in general population. Thus, this statement assume even more importance talking about HTx patients, who are already at higher risk of cardio-vascular complications. LARN references recommend a %E from SFA intake <10% (96), and patients of our study not only significantly decreased their %E from SFA over the study period, but they also reached this recommended value at the final timepoint, switching from a baseline mean %E from SFA of 13.0±2.1% to a final 9.6±1.5 ($p=0.001$). Nevertheless, the median %E from PUFA remained below the recommended range of 5 – 10% (96), underling the need to keep encouraging these patients to consume more fish and nuts, foods rich in omega-3 PUFA (31). To note, the result observed could be underestimated because of the presence of the missing values in the food composition tables (118) used to analyse food diaries, as specified in the "study limitations". Anyway, most of the HTx patients of our centre are preventively treated with omega-3 supplementation. MUFA intake, instead, remained adequately provided over the study period, proved by the increased olive oil consumption observed in the final FFQ. Finally, also cholesterol intake, already below the limit of 300mg from LARN recommendations (96) at baseline, remained in the adequate range of consumption with, rather, a reduction in the final timepoint. Evidence from observational studies conducted in several countries generally does not indicate a significant association between dietary cholesterol and CVD risk. Although meta-analyses of intervention studies differ in their findings, most associate intakes of cholesterol exceeding current average levels with elevated total or low-density lipoprotein cholesterol concentrations (119). Thus, the advice remains to prefer healthy dietary patterns, like MedDiet, which naturally contains low-cholesterol foods. As a matter of fact, the main strength of MedDiet is that plant foods constitute the core of its daily intake, whereas foods from animal are typically limited. Consuming the recommended servings of fruits, vegetables and legumes, it also provides an adequate intake of essential micronutrients and vitamins, of which these foods are rich. This is very important for HTx patients, who are chronically exposed to the side effects of immunosuppressive therapy and many other treatments. Among all, Cyclosporine has significant effects on the metabolism and disposition of several biomolecules. Hypomagnesemia is a very frequently observed electrolyte disorder caused by this calcineurin inhibitor (5), and oral supplementation of magnesium is routinely prescribed to HTx patients. Moreover, we noted an increase in mean Mg intake over the study period, reaching the

target of 170mg from LARN recommendations (96) in the final timepoint. This means that patients, through diet, were able to cover the required need of Mg, despite the oral supplementation.

Calcineurin inhibitors, together with glucocorticoids, have also a role in boon loss, a common and debilitating problem after HTx. Most bone loss occurs in the first 6 to 12 months after transplantation when steroid doses are highest (15). Optimal treatment of osteoporosis requires adequate Ca (1000 to 1500 mg/d) and VitD (400-1000 IU) intake (120). From food diaries analyses, a significant reduction in Ca intake appeared at all timepoints, with all values lower than the recommendations (96). However, these resulted values are likely to be underestimated, because drinking water was not properly reported by patients in the food diaries, as mentioned regarding food diary limitations in the "study limitations". Water, both tap and bottled, is, in fact, a significant source of Ca (121-123). Different speech for VitD, which values again did neither increase nor reach the indications of LARN (96) at any timepoints, but all the patients were treated with oral supplementation. Moreover, diet does not represent the principal source of VitD, which is instead mainly synthetized with the sun exposition.

Cyclosporine seems to play a role also in the homeostasis of homocysteine, which is altered in solid organ transplant recipients, likely by inducing renal insufficiency (120). Elevated homocysteine levels have been noted in more than half of HTx recipients and have been associated with the development of atherosclerosis in non-transplant population, as well as low vitamin B6 levels (124). However, Nahlawi et al., did not find the same association in their 160 HTx patients with high homocysteine level, while both falling creatinine clearance and vitamin B6 deficiency were found to be predictive of a worse outcome, similar to findings in the general population (124). In our study population, mean VitB6 value was already in the recommended range at the baseline timepoint, and remained adequately covered over the study period. The same observation also applied to VitB2, niacin and B12. Otherwise, VitB1 and Biotin, although an improvement between baseline and intermediate timepoints, did not reach the adequate intake (96). As reported in the Guidelines from CREA, legumes are rich in both VitB1 and Biotin (95), and this could explain the slight deficiency observed for these two micronutrients. Legumes are, indeed, among the less consumed food in our HTx population, as reported in Aim 2, and confirmed in this study, as demonstrated by FFQ results. The consumption increased over the study period, but apparently not enough to provide an adequate intake of the mentioned micronutrients. Actually, most of the patients, at baseline, consumed legumes erroneously as a side dish, like vegetables, rather than a proper second dish, like meat, eggs or fish. Here is where a nutritional intervention proves its usefulness shifting patients towards healthier dietary habits through a correct education. This also applies to iron intake, which has always been

believed to be found mainly in red meat. However, also legumes are rich in iron, as well as in proteins, other micronutrients like zinc and copper, fibres and group B vitamins, as already mentioned (95). Iron is a critical mineral for maintaining homeostasis in the human body. It is essential for several cellular processes and its deficiency is an important predictor of cardiovascular events and all-cause mortality (125). In our study population, mean iron intake was already within the recommended range (96) at the baseline timepoint, and remained adequate over the study period. Another reason to encourage a major consumption of legumes are the mean values of both zinc and copper, neither of them reaching the recommended range of LARN (96) at the final timepoint. Zinc is the second most abundant transition metal in the body after iron. Through its presence in the structure of various enzymes and proteins, zinc plays a major role in normal cell structure and catalytic function, especially in the immune and central nervous systems. It is also critical to growth, cell division and repair, wound healing, energy producing functions, carbohydrate catabolism, hemostasis, and thrombosis (125). Copper is essential for enzyme function, to mitochondrial respiration and iron absorption, and has an important antioxidant role (125). Among these transition metals, also Se has a pivotal role in the human body. Through proteins known as selenoprotein, Se prevents oxidative stress, facilitates thyroid hormone metabolism, and maintains antioxidant enzyme (125). In our study population, although an increase was observed from baseline to meeting 3, the final value did not achieve the adequate indication from LARN (96).

Along with Se, also Vitamins C and E are universally recognized for their anti-oxidative effects (75). Both fruits and vegetables are rich in Vitamins A, C and E, and in our study population the intake of the first two appeared increased over the study period and in accordance with the LARN recommendations (96). Also VitE intake has risen almost significantly from the baseline value to the last one ($p=0.062$), although still not reaching the range of adequacy.

Finally, we must always remember that there is neither nor can exist in the current state of knowledge a nutrient or a component that alone, outside of a proper diet, is able to protect against cardiovascular diseases and oxidative stress. Foods are not "sums of molecules" but are elements with which, depending on the components, a correct diet is built, essential for the prevention and defence of health.

Since the goal of this dietary intervention was to optimize the nutritional status of the patients involved, rather than focusing in obtaining significative changes, this study provide evidence that the implementation of a structured and personalized healthy eating programme is feasible in HTx populations and exerts many beneficial effects. A timely monitoring of the progresses may be

effective to consolidate the provided dietary indications and reach an adequate consumption of all macronutrients and micronutrients intake.

6 Study Limitations

An experimental study involving patients is always a challenge, but an experimental study involving immunosuppressant patients during a virus pandemic is a fight with unequal weapons.

The main limitation of this study was the unexpected variable of the COVID-19 pandemic. It took more than a year to involve all the necessary professional personnel, to create a collaboration with the Post-Graduate School in Hygiene and Preventive Medicine, and finally design a structured and feasible dietary program. Once obtained the Ethic Committee of Friuli Venezia Giulia region approval, we could not begin the patients enrolment because the COVID-19 pandemic was raging, as still rages today. Postponing the start of the study and slowing down the enrolments, led to the greater limitation of this study, the small sample size. However, all the evidences observed were coherent with an improved adherence to the Mediterranean diet, as reported in many other studies on both transplanted patients (59-61) and general population (68, 69). More data are needed on larger population and longer follow-up to confirm the results obtained so far.

Despite the randomised design of the two groups, there were a tendency to older and less recently transplanted patients in the control group. Potentially, increasing the sample size, this tendency may be resolved.

Physical activity level is inextricably linked with composite health outcomes. Although it was evaluated with IPAQ and encourage during the scheduled meetings, a structured program is need to promote more effectively physical activity in heart transplanted patients, in order to obtain a synergic benefits. Moreover, more specific tools are needed to evaluate more efficiently the physical activity baseline level and improvements of patients, for example through a 6 Minutes Walking Test (6MWT) or a CardioPulmonary Exercise Test (CPET).

As regard food diaries analysis, some dietary records were incomplete or inaccurately completed. In case of missing information, standard recipes and standard portion sizes were used. Moreover, added salt was not accurately reported by all subjects, and no information on its iodization has been provided. Similarly, water consumption was sporadically reported, leading to possible underestimation of minerals, especially of calcium. An additional source of underestimation of nutrients - common to other studies - included missing data in the food composition tables (118). Additionally, the use of nutritional labels for the conversion of complex commercial products may have led to inaccurate estimates of a few macronutrients and/or underestimation of micronutrient intakes.

Finally, the study lacked in some evaluation of biomarkers on insulin resistance, systemic inflammation and hormonal profile, which could have helped to clarify the overall benefit of the dietary program. These additional analyses were planned in the original project but, because of the small sample size, we were not able to reach the adequate number of collected samples.

7 Conclusions

Heart transplanted patients represent a unique population under many aspects. From a clinical point of view, they need a lifelong monitoring to control multiple risk factors that involves a multi-disciplinary dedicated team. However, above all, from a human point of view, they symbolise the richest gift of all: a new chance of life, a rebirth. For these reasons, it is of paramount importance to make any possible effort in optimising their health status and minimising future transplant-related complications. Despite many advances in patients' management and pharmacological treatment, metabolic syndrome still represents a real burden in heart transplanted recipients, leading to a whole series of related complications that may severely affect their long-term outcome. Among all, a change in dietary habits towards a healthier diet, like Mediterranean pattern, seems to constitute an effective strategy to control the development of cardiovascular risk factors and metabolic syndrome itself. Unfortunately, dietary programs for the long-term period after heart transplantation are not yet adequately provided in the routine follow-up of these patients.

In Aim 1 of this research project, we demonstrated the high prevalence of metabolic syndrome among cardiac transplanted patients at the University Hospital of Udine. Moreover, the early development of metabolic syndrome, both before and within 1 year of transplantation, were associated to a worst survival and to a higher risk to develop cardiac allograft vasculopathy.

Since its proved beneficial effects on cardiovascular risk factors, in Aim 2 we analysed the actual adherence to Mediterranean diet in a sample of cardiac transplanted patients, observing an overall weak adherence and an inadequate consumption of many healthy foods typical of this dietary pattern.

Finally, in Aim 3 we designed a structured and personalized dietary education for cardiac transplanted patients and we evaluated the effects of this intervention both within the group of patients enrolled and compared to a control group. We highlighted a significant improved in adherence to Mediterranean diet in patients of intervention group, as well as improvements in body composition, blood pressure and dietary habits. In particular, we observed a more balanced macronutrients intake, a reduced consumption in saturated fatty acids and sugars, and a mostly sufficient intake of the essential micronutrients.

Concluding, we proved that the implementation of a structured and personalized dietary programme is feasible in heart transplanted population and exerts many beneficial effects. Moreover, the long-term advantages of promoting healthy eating positively decrease downstream health care costs.

This pilot study aimed not only to detect the potentially benefits reached with a nutritional education in cardiac transplanted patients, but also possible difficulties in organization, patient compliance or analysis methods. Thanks to the obtained results, our goal will be to offer a nutritional education to all cardiac transplanted patients at the University Hospital of Udine, with the ambitious intent to insert this treatment as a routine prescription in the standard follow-up of these patients. This objective will be possible with the institution of a dedicated nutritional ambulatory in the Heart Transplant ambulatory at the Cardiothoracic Department of the University Hospital of Udine, in order to concentrate, in one site, all the professional figures involved in the cardiac transplanted patients follow-up.

8 Bibliography

1. ISHLT: Heart Transplantation report - Adult Recipients. 2019.

2. Collaborators GDaIIaP: Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet 2018;392.

3. McCartney S, Patel C, Del Rio J: Chapter 9. Long Term Outcomes and Management of the Heart Transplant Recipient. Best Practice & Research Clinical Anaesthesiology: Elsevier; 2017.

4. Stehlik J, Kobashigawa J, Hunt S, Reichenspurner H, Kirklin J: Honoring 50 Years of Clinical Heart Transplantation in Circulation: In-Depth State-of-the-Art Review. Circulation 2018;137.

5. Lindenfeld J, Miller G, Shakar S, et al.: Drug Therapy in the Heart Transplant Recipient. Part II: Immunosuppressive Drugs. Circulation 2004;110.

6. Lechiancole A, Vendramin I, Sponga S, et al.: Influence of donor-transmitted coronary artery disease on long-term outcomes after heart transplantation - a retrospective study. TRANSPLANT INTERNATIONAL 2020.

7. Lindenfeld J, Miller G, Shakar S, et al.: Drug Therapy in the Heart Transplant Recipient. Part I: Cardiac Rejection and Immunosuppressive Drugs. Circulation 2004;110.

8. Bennett A, Ventura H: Hypertension in Patients with Cardiac Transplantation. Med Clin North Am 2017;101.

9. Vega E, Schroder J, Nicoara A: Postoperative management of heart transplantation patients. Best Practice & Research Clinical Anaesthesiology 2017;31.

10. Entwistle T: Implementing a Healthy Eating Strategy after Heart and Lung Transplantation: A Randomised Controlled Feasibility Study. 2016.

11. Potena L, Zuckermann A, Barberini F, Aliabadi-Zuckermann A: Complications of Cardiac Transplantation. Curr Cardiol Rep 2018;20.

12. Stewart S, Winters G, Fishbein M, et al.: Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant 2005;24.

13. Hamour I, Omar F, Lyster H, Palmer A, Banner N: Chronic kidney disease after heart transplantation. Nephrol Dial Transplant 2009;24:1655-62.

14. Taylor DO EL, Boucek MM: The Registry of the International Society for Heart and Lung Transplantation: twenty-first International Society for Heart and Lung Transplantation: twenty-first. J Heart and Lung Transpl 2004;23:796–803.

15. Lindenfeld J, Miller G, Shakar S, et al.: Drug Therapy in the Heart Transplant Recipient. Part III: Common Medical Problems. Circulation 2005;111.

16. Valantine H: Cardiac Allograft Vasculopathy After Heart Transplantation: Risk Factors and Management. J Heart Lung Transplant 2004;23.

17. Williams J, Lund L, LaManca J, et al.: Excessive Weight Gain in Cardiac Transplant Recipients. J Heart Lung Transplant 2006;25:36-41.

18. Ward H: Nutritional and Metabolic Issues in Solid Organ Transplantation: Targets for Future Research. Journal of Renal Nutrition 2009;19:111-22.

19. Zhao T, Zhao Y, Zong A, Tang Y, Shi X, Y Z: Association of body mass index and fasting plasma glucose concentration with post-transplantation diabetes mellitus in Chinese heart transplant recipients. J Int Med Res 2020;48.

20. Amarelli C, Buonocore M, Romano G, Maiello C, De Santo L: Nutritional issues in heart transplant candidates and recipients. Front Biosci (Elite Ed) 2012;4.

21. de Souza-Neto J, de Oliveira Ĺ, Lima-Rocha H, Oliveira-Lima J, Bacal F: Hypertension and arterial stiffness in heart transplantation patients. Clinics (Sao Paulo) 2016;71.

22. Shiba N, Chan M, Kwok B, et al.: Analysis of Survivors More Than 10 Years After Heart Transplantation in the Cyclosporine Era: Stanford Experience. J Heart Lung Transplant 2004;23.

23. Akhlaghi F, Jackson C, Parameshwar J, Sharples L, Trull A: Risk factors for the development and progression of dyslipidemia after heart transplantation. Transplantation 2002;73.

24. Zielińska K, Kukulski L, Wróbel M, Przybyłowski P, Zakliczyński M, Strojek K: Prevalence and Risk Factors of New-Onset Diabetes After Transplantation (NODAT). Ann Transplant 2020;25.

25. Dandel M, Lehmkuhl H, Knosalla C, Hetzer R: Impact of different long-term maintenance immunosuppressive therapy strategies on patients' outcome after heart transplantation. Transpl Immunol 2010;23.

26. Cordero Fort A, Gavira J, Algeria-Barrero E, et al.: Prevalence of Metabolic Syndrome in Heart Transplant Patients: Role of Previous Cardiopathy and Years Since the Procedure—the TRACA Study. J Heart Lung Transplant 2006;25.

27. Bozkurt B, Aguilar D, Deswal A, et al.: Contributory Risk and Management of Comorbidities of Hypertension, Obesity, Diabetes Mellitus, Hyperlipidemia, and Metabolic Syndrome in Chronic Heart Failure. A Scientific Statement From the American Heart Association. Circulation 2016;134.

28. Sorice G, Muscogiuri G, Mezza T, Prioletta A, Giaccari A: Metabolic Syndrome in Transplant Patients: An Academic or a Health Burden? Transplantation Proceedings 2011;43.

29. Sanchez-Gomez J, Martinez-Dolz L, Sanchez-Lazaro I, et al.: Influence of Metabolic Syndrome on Development of Cardiac Allograft Vasculopathy in the Transplanted Heart. Transplantation 2012;93.

30. Mendrick D, Diehl A, Topor L, et al.: Metabolic Syndrome and Associated Diseases: From the Bench to the Clinic. TOXICOLOGICAL SCIENCES 2018;162:36-42.

31. Finicelli M, Squillaro T, Di Cristo F, et al.: Metabolic syndrome, Mediterranean diet, and polyphenols: Evidence and perspectives. J Cell Physiol 2019;234.

32. Boden G, Shulman G: Free fatty acids in obesity and type 2 diabetes: Defining their role in the development of insulin resistance and beta-cell dysfunction. European Journal of Clinical Investigation 2002;32.

33. Cornier M, Dabelea D, Hernandez T, et al.: The metabolic syndrome. Endocr Rev 2008;29.

34. Hirabara S, Silveira L, Abdulkader F, Carvalho C, Procopio J, Curi R: Time-dependent effects of fatty acids on skeletal muscle metabolism. Journal of Cellular Physiology 2007;210.

35. Muniyappa R, Sowers J: Role of insulin resistance in endothelial dysfunction. Reviews in Endocrine and Metabolic Disorders 2013;14.

36. Kim J, Montagnani M, Koh K, Quon M: Reciprocal relationships between insulin resistance and endothelial dysfunction: Molecular and pathophysiological mechanisms. Circulation 2006;113.

37. Smith R, Soeters M, Wüst R, Houtkooper R: Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. Endocrine Reviews 2018;39.

38. Jørgensen W, Rud K, Mortensen O, Frandsen L, Grunnet N, Quistorff B: Your mitochondria are what you eat: a high-fat or a high-sucrose diet eliminates metabolic flexibility in isolated mitochondria from rat skeletal muscle. Physiol Rep 2017;5.

39. Park M, Sweeney G: Direct effects of adipokines on the heart: focus on adiponectin. Heart Fail Rev 2013;18.

40. Leroith D: Pathophysiology of the metabolic syndrome: implications for the cardiometabolic risks associated with type 2 diabetes. Am J Med Sci 2012;343.

41. Ando K, Fujita T: Metabolic syndrome and oxidative stress. Free Radical Biology and Medicine 2009;47.

42. Di Daniele N, Noce A, Vidiri M, Moriconi E, Marrone G, Annicchiarico-Petruzzelli M: Impact of Mediterranean diet on metabolic syndrome, cancer and longevity. Oncotarget 2017;8.

43. Bays H, González-Campoy J, Bray G, et al.: Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. Expert Rev Cardiovasc Ther 2008;6.

44. Monteiro R, Azevedo I: Chronic inflammation in obesity and the metabolic syndrome. Mediators of Inflammation 2010.

45. Tangvarasittichai S, Pingmuanglaew P, Tangvarasittichai O: Association of elevated serum lipoprotein(a), inflammation, oxidative stress and chronic kidney disease with hypertension in non-diabetes hypertensive patients. Indian Journal of Clinical Biochemistry 2016;31.

46. Martinez-Dolz L, Sanchez-Lazaro I, Almenar-Bonet L, et al.: Metabolic syndrome in heart transplantation: impact on survival and renal function. Transplant International 2013;26.

47. Sanchez-Lazaro I, Sanchez-Gomez J, Almenar-Bonet L, et al.: Long-Term Prognostic Implications of Metabolic Syndrome in Heart Transplant Recipients. Transplantation Proceedings 2011;43.

48. Valantine H, Rickenbacker P, Kemna M, et al.: Metabolic abnormalities characteristic of dysmetabolic syndrome predict the development of transplant coronary artery disease: a prospective study. Circulation 2001;103.

49. Biadi O, Potena L, Fearon W, et al.: Interplay Between Systemic Inflammation and Markers of Insulin Resistance in Cardiovascular Prognosis After Heart Transplantation. J Heart Lung Transplant 2007;26.

50. Raichlin E, McConnell J, Lerman A, Kremers W, Edwards B, Kushwaha S: Systemic inflammation and metabolic syndrome in cardiac allograft vasculopathy. J Heart Lung Transplant 2007;26.

51. Festa A, Hanley A, Tracy R, D'Agostino RJ, Haffner S: Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulin secretion. Circulation 2003;108.

52. Pickup J, Crook M: Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998;41.

53. Lee J, Walker M, Bourdillon M, et al.: Cojoint Associations of Adherence to Physical Activity and Dietary Guidelines With Cardiometabolic Health: The Framingham Heart Study. J Am Heart Assoc 2021;10.

54. WHO: Diet, nutrition and the prevention of chronic diseases. 2003;916.

55. Bellinghieri G, Bernardi A, Piva M, et al.: Metabolic syndrome after kidney transplantation. J Ren Nutr 2009;19.

56. Zaffari D, Losekann A, Santos A, Manfroi W, Bittar A, Keitel E: Effectiveness of diet in hyperlipidemia in renal transplant patients. Transplant Proc 2004;36.

57. Goodman D, Hulley S, Clark L, et al.: Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch Intern Med 1988;148.

58. Guida B, Perrino NR, Laccetti R, et al.: Role of dietary intervention and nutritional follow-up in heart transplant recipients. Clin Transplant 2009;23:101-7.

59. Entwistle T, Green A, Fildes J, K M: Adherence to Mediterranean and low-fat diets among heart and lung transplant recipients: a randomized feasibility study. Nutrition Journal 2018;17.

60. Entwistle TR, Miura K, Keevil BG, et al.: Modifying dietary patterns in cardiothoracic transplant patients to reduce cardiovascular risk: The AMEND-IT Trial. Clin Transplant 2021;35:e14186.

61. Salen P, de Lorgeril M, Boissonnat P, et al.: Effects of a French Mediterranean diet on heart transplant recipients with hypercholesterolemia. Am J Cardiol 1994;73:825-7.

62. Moro E: La dieta mediterranea. Mito e storia di uno stile di vita: Il Mulino; 2014.

63. Willett W, Sacks F, Trichopoulou A, et al.: Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 1995;61.

64. IFMED: New Pictorial Proposal for an Environmental, Sustainable Mediterranean Diet. 2016.

65. Piepoli M, Hoes A, Agewall S, et al.: 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. Eur Heart J 2016;37.

66. Zeltzer S, Taylor D, Wilson Tang W: Long-term Dietary Habits and Interventions in Solid Organ Transplantation. J Heart Lung Transplan 2015;34.

67. Sakhaei R, Shahvazi S, Mozaffari-Khosravi H, Samadi M, Khatibi N: The dietary approaches to stop hypertension (DASH)-style diet and an alternative Mediterranean diet are differently associated with serum inflammatory markers in female adults. Food and Nutrition Bulletin - SAGE 2018.

68. Estruch R, Ros E, Salas-Salvado J, et al.: Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. New England Journal of Medicine 2013;368.

69. Esposito K, Marfella R, Ciotola M, et al.: Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004;292.

70. Kastorini C, Milionis H, Esposito K, Giugliano D, Goudevenos J, Panagiotakos D: The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. J Am Coll Cardiol 2011;57.

71. Papadaki A, Nolen-Doerr E, Mantzoros C: The Effect of the Mediterranean Diet on Metabolic Health: A Systematic Review and Meta-Analysis of Controlled Trials in Adults. Nutrients 2020;12.

72. Bakaloudi D, Chrysoula L, Kotzakioulafi E, Theodoridis X, Chourdakis M: Impact of the Level of Adherence to Mediterranean Diet on the Parameters of Metabolic Syndrome: A Systematic Review and Meta-Analysis of Observational Studies. Nutrients 2021;13.

73. Godos J, Zappalà G, Bernardini S, Giambini I, Bes-Rastrollo M, Martinez-Gonzalez M: Adherence to the Mediterranean diet is inversely associated with metabolic syndrome occurrence: a meta-analysis of observational studies. Int J Food Sci Nutr 2017;68.

74. Kastorini C, Panagiotakos D, Chrysohoou C, et al.: Metabolic syndrome, adherence to the Mediterranean diet and 10-year cardiovascular disease incidence: The ATTICA study. Atherosclerosis 2016;246.

75. Patel J: Vitamin therapy after heart transplantation. Expert Review of Cardiovascular Therapy 2015;13.

76. Mthembu S, Dludla P, Ziqubu K, et al.: The Potential Role of Polyphenols in Modulating Mitochondrial Bioenergetics within the Skeletal Muscle: A Systematic Review of Preclinical Models. Molecules 2021;26.

77. Amiot M, Riva C, Vinet A: Effects of dietary polyphenols on metabolic syndrome features in humans: a systematic review. Obes Rev 2016;17.

78. Abe R, Beckett J, N, A, Rochier A, Yamashita N, Sumpio B: Olive oil polyphenol oleuropein inhibits smooth muscle cell proliferation. European Journal of Vascular and Endovascular Surgery 2011;41.

79. López-Miranda J, Pérez-Jiménez F, Ros E, De Caterina R, Badimón L, Covas M: Olive oil and health: Summary of the II international conference on olive oil and health consensus report. Nutrition, Metabolism, and Cardiovascular Disease 2010;20.

80. Chen M, Hou P, Zhou M, et al.: Resveratrol attenuates high-fat diet-induced non-alcoholic steatohepatitis by maintaining gut barrier integrity and inhibiting gut inflammation through regulation of the endocannabinoid system. Clin Nutr 2020;39.

81. Truong V, Jun M, Jeong W: Role of resveratrol in regulation of cellular defense systems against oxidative stress. Biofactors 2018;44.

82. Esposito K, Nappo F, Giugliano F, et al.: Meal modulation of circulating interleukin 18 and adiponectin concentrations in healthy subjects and in patients with type 2 diabetes mellitus. Am J Clin Nutr 2003;78.

83. Augustin L, Kendall C, Jenkins D, et al.: Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). Nutr Metab Cardiovasc Dis 2015;25.

84. Salmerón J, Ascherio A, Rimm E, et al.: Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care 1997;20.

85. Jenkins D, Kendall C, Augustin L, et al.: Effect of legumes as part of a low glycemic index diet on glycemic control and cardiovascular risk factors in type 2 diabetes mellitus: a randomized controlled trial. Arch Intern Med 2012;172.

86. Nazari J, Yadegari N, Khodam S, Almasi-Hashian A, Amini S: Effect of Consumption of Whole-Wheat Breads on FBS, HbA1c, and Blood Lipids in Patients with Type 2 Diabetes. Prev Nutr Food Sci 2021;26.

87. Albracht-Schulte K, Kalupahana N, Ramalingam L, et al.: Omega-3 fatty acids in obesity and metabolic syndrome: A mechanistic update. Journal of Nutritional Biochemistry 2018;58.

88. Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, Hensler M: n-3 Fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. Diabetologia 2009;52.

89. Mehra MR C-LM, Dipchand A, Ensminger SM, Hiemann, NE, Kobashigawa J, Madsen J, Parameshwar J, Starling R, Uber P: International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. J Heart Lung Transplant 2010;29.

90. Gragnarella P, Dragà D, Misotti A, et al.: Validation of a short questionnaire to record adherence to the Mediterranean diet: An Italian experience. Nutrition, Metabolism and Cardiovascular Disease 2018;28.

91. Prete M, Luzzetti A, Augustin L, et al.: Changes in Lifestyle and Dietary Habits during COVID-19 Lockdown in Italy: Results of an Online Survey. Nutrients 2021;13.

92. Hagströmer M, Oja P, Sjöström M: The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. Public Health Nutr 2006;9.

93. Trichopoulou A, Bamia C, Trichopoulos D: Anatomy of health effects of Mediterranean diet: Greek EPIC prospective cohort study. BMJ 2009;338.

94. Trichopoulou A, Bamia C, Trichopoulos D: Mediterranean diet and survival among patients with coronary heart disease in Greece. Arch Intern Med 2005;165.

95. (CREA) CdrAeN: Linee guida per una sana alimentazione 2018; 2018.

96. (SINU) SIdNU: LARN. Livelli di Assunzione di Riferimento di Nutrienti ed energia per la popolazione italiana. IV edizione: SICS; 2014.

97. Guida B, Perrino N, Laccetti R, et al.: Role of dietary intervention and nutritional follow-up in heart transplant recipients. Clin Transplant 2009;23.

98. R. L, M. RJ, F. B, et al.: Relation of Prolonged Pacemaker Dependency After Cardiac Surgery to Mortality. THE AMERICAN JOURNAL OF CARDIOLOGY 2021;138:66-71.

99. Bibas L, Saleh E, Al-Kharji S, et al.: Muscle Mass and Mortality After Cardiac Transplantation. Transplantation 2018;102.

100. Fernández de la Puebla R, Fuentes F, Pérez-Martinez P, et al.: A reduction in dietary saturated fat decreases body fat content in overweight, hypercholesterolemic males. Nutr Metab Cardiovasc Dis 2003;13.

101. Álvarez-Pérez J, Sánchez-Villegas A, Díaz-Benítez E, et al.: Influence of a Mediterranean Dietary Pattern on Body Fat Distribution: Results of the PREDIMED-Canarias Intervention Randomized Trial. J Am Coll Nutr 2016;35.

102. Janssen I, Katzmarzyk P, Ross R: Waist circumference and not body mass index explains obesity-related health risk. Am J Clin Nutr 2004;79.

103. Nordmann A, Suter-Zimmermann K, Bucher H, et al.: Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. Am J Med 2011;124.

104. Mozaffarian D, Fahimi S, Singh G, et al.: Global sodium consumption and death from cardiovascular causes. N Engl J Med 2014;371.

105. Braith R, Mills RJ, Wilcox C, Davis G, Wood C: Breakdown of blood pressure and body fluid homeostasis in heart transplant recipients. J Am Coll Cardiol 1996;27.

106. Denic A, Glassock R, Rule A: Structural and Functional Changes With the Aging Kidney. Adv Chronic Kidney Dis 2016;23.

107. DiNicolantonio J, Lucan S: The wrong white crystals: not salt but sugar as aetiological in hypertension and cardiometabolic disease. Open Heart 2014;1.

108. Organization WH: World Health Organization (2015) Guideline: sugars intake for.

109. Talayero B, Sacks F: The role of triglycerides in atherosclerosis. Curr Cardiol Rep 2011;13.

110. Vitale M, Masulli M, Rivellese A, et al.: Influence of dietary fat and carbohydrates proportions on plasma lipids, glucose control and low-grade inflammation in patients with type 2 diabetes-The TOSCA.IT Study. Eur J Nutr 2016;55.

111. Vega G, Barlow C, Grundy S, Leonard D, DeFina L: riglyceride-to-high-density-lipoprotein-cholesterol ratio is an index of heart disease mortality and of incidence of type 2 diabetes mellitus in men. J Investig Med 2014;62.

112. Association. AD: Classification and diagnosis of diabetes. 2015;38.

113. Rosettenstein K, Viecelli A, Yong K, et al.: Diagnostic Accuracies of Glycated Hemoglobin, Fructosamine, and Homeostasis Model Assessment of Insulin Resistance in Predicting Impaired Fasting Glucose, Impaired Glucose Tolerance, or New Onset Diabetes After Transplantation. Transplantation 2015;100.

114. Julibert A, Bibiloni M, Bouzas C, et al.: Total and Subtypes of Dietary Fat Intake and Its Association with Components of the Metabolic Syndrome in a Mediterranean Population at High Cardiovascular Risk. Nutrients 2019;11.

115. Siri-Tarino P, Sun Q, Hu F, Krauss R: Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr 2010;91.

116. Hooper L, Martin N, Abdelhamid A, GD S: Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst Rev 2015;6.

117. Mazidi M, Mikhailidis D, Sattar N, et al.: Association of types of dietary fats and all-cause and cause-specific mortality: A prospective cohort study and meta-analysis of prospective studies with 1,164,029 participants. Clin Nutr 2020;39.

118. Concina F, Carletti C, Pani P, Knowles A, Barbone F, Parpinel, M: Development of a food composition database to study complementary feeding: An Italian experience. Journal of Food Composition and Analysis 2015;46.

119. Carson J, Lichtenstein A, Anderson C, et al.: Dietary Cholesterol and Cardiovascular Risk: A Science Advisory From the American Heart Association. Circulation 2020;141.

120. Posada-Moreno P, Ortuno-Soriano I, Zaragoza-Garcia I, et al.: Nutritional Intervention in Heart Transplant Recipients – Dietary Recommendations. Cardiac Transplantation: InTech; 2012.

121. Martínez-Ferrer A, Peris P, Reyes R, Guañabens N: Intake of calcium, magnesium and sodium through water: health implications. Med Clin 2008;131.

122. Azoulay A, Garzon P, Eisenberg M: Comparison of the mineral content of tap water and bottled waters. J Gen Intern Med 2001;16.

123. Cormick G, Lombarte M, Minckas N, et al.: Contribution of calcium in drinking water from a South American country to dietary calcium intake. BMC Res Notes 2020;13.

124. Nahlawi M, Seshadri N, Boparai N, et al.: Usefulness of plasma vitamin B(6), B(12), folate, homocysteine, and creatinine in predicting outcomes in heart transplant recipients. Am J Cardiol 2002;89.

125. Mohammadifard N, Humphries K, Gotay C, et al.: Trace minerals intake: Risks and benefits for cardiovascular health. Crit Rev Food Sci Nutr 2019;59.

9 Appendix A. Food Frequency questionnaire

NOME E COGNOME _____

ID _____ **Data di nascita** ___/___/_____ **Data di compilazione** ___/___/_____

Peso _____ **kg** _____ - **Altezza** _____ **cm**

Buongiorno, Vorremmo farle alcune domande sulla sua dieta abituale. Indichi, con una crocetta "X", il numero di porzioni normalmente consumate per i 15 alimenti o gruppi di alimenti elencati nella tabella sottostante.

Si aiuti con le porzioni di riferimento per identificare la sua frequenza di consumo giornaliera, per gli alimenti elencati dalla domanda n. 1 alla n. 8 e la sua frequenza di consumo settimanale per gli alimenti dalla n. 9 alla n. 15.

Se abitualmente consuma una porzione molto piccola o molto grande (rispetto alla porzione di riferimento) dimezzi o raddoppi la frequenza di consumo. Per esempio se normalmente beve mezzo litro di vino al giorno (corrispondenti a circa 4 bicchieri), la frequenza da segnare in tabella sarà "3-4" porzioni al giorno.

E' molto importante che risponda a tutte le domande. Nel caso non consumasse qualche alimento, ricordi di fare una crocetta su "mai o raramente".

Con quale frequenza consuma normalmente una porzione dei seguenti alimenti?

ALIMENTI	PORZIONE	FREQUENZA DI CONSUMO AL GIORNO				
		Mai o raramente	Meno di 1 volta /giorno	1 volta /giorno	2 volte /giorno	≥ 3 volte /giorno
1. Pasta o riso di tipo integrale	80 gr	☐	☐	☐	☐	☐
2. Verdura tutti i tipi (sia cruda che cotta)	200 gr (80 gr insalata)	☐	☐	☐	☐	☐
3. Frutta tutti i tipi, anche la spremuta fresca	150 gr	☐	☐	☐	☐	☐
4. Latte e yogurt	1 bicchiere/ vasetto (125 gr)	☐	☐	☐	☐	☐
		Mai o raramente	Meno di 1 volta /giorno	1-2 volte /giorno	3-4 volte /giorno	≥ 5 volte /giorno
5. Pane e fette di tipo integrale	1-2 fette (50 gr)	☐	☐	☐	☐	☐
6. Olio di oliva per cucinare e condire	1 cucchiaio (10 ml)	☐	☐	☐	☐	☐
7. Burro, margarina o panna da cucina per cucinare	1 noce (10 gr)	☐	☐	☐	☐	☐
8. Vino (bianco e rosso)	1 bicchiere (125 ml)	☐	☐	☐	☐	☐
ALIMENTI	**PORZIONE**	**FREQUENZA DI CONSUMO ALLA SETTIMANA**				
		Mai o raramente	Meno di 1 volta /sett	1-3 volte /sett	4-6 volte /sett	≥ 7 volte /sett
9. Carne rossa (bovino, vitello, maiale), affettati e salumi	100 gr (carne) 50 gr (salumi)	☐	☐	☐	☐	☐
10. Carne bianca (pollo, tacchino, coniglio)	100 gr	☐	☐	☐	☐	☐
11. Bevande dolci o gassate (tipo coca-cola, aranciata, gassosa, ecc)	1 bicchiere (200 ml)	☐	☐	☐	☐	☐
12. Dolci o pasticcini (non fatti in casa), come torte, biscotti, creme o dolci al cucchiaio	100 gr	☐	☐	☐	☐	☐
		Mai o raramente	Meno di 1 volta /sett	1 volta /sett	2-3 volte /sett	≥ 4 volte /sett
13. Pesce (fresco o surgelato) o frutti di mare	150 gr (pesce) 50 gr (frutti di mare)	☐	☐	☐	☐	☐
14. Frutta secca (noci, mandorle, nocciole)	1 pugno (30 gr)	☐	☐	☐	☐	☐
15. Legumi (ceci, lenticchie, piselli, fagioli)	50 gr (secchi) 150 gr (scatola / freschi)	☐	☐	☐	☐	☐

QuestionarioDietaMV5

Grazie mille per la sua disponibilità!

10 Appendix B. International Physical Activity Questionnaire (IPAQ)

Questionario sull'attività fisica quotidiana (IPAQ)

ISTRUZIONI PER LA COMPILAZIONE

- Questo questionario misura il tipo e la quantità di attività fisica che lei fa normalmente. Le domande si riferiscono all'attività svolta negli ultimi 7 giorni **al lavoro**, **per spostarsi** da un posto all'altro e **nel tempo libero**
- Per attivita' fisica **MODERATA** si intende un'attivita' che richiede uno sforzo fisico moderato e che la costringe a **respirare con un ritmo solo moderatamente piu' elevato del normale** (durante tale attività non riuscirebbe a cantare ma le sarebbe ancora possibile parlare).
- Per attivita' fisica **INTENSA** si intende un'attivita' che richiede uno sforzo fisico elevato e che la costringe a **respirare con un ritmo molto piu' elevato del normale** (durante tale attività si suda e non si riesce a parlare).
- Nel rispondere alle domande, tenga conto solo di quelle attivita' che l'hanno impegnata per **almeno 10 minuti** consecutivi.

Attività intense

1a Negli ultimi 7 giorni, per quanti giorni ha compiuto attivita' fisiche **INTENSE**, come ad esempio sollevamento di pesi, lavori pesanti in giardino, attivita' aerobiche come corse o giri in bicicletta a velocità sostenuta?

_____ giorni alla settimana

Nemmeno uno → (vada alla domanda **2a**)

1b Quanto tempo in totale, normalmente, lei ha trascorso compiendo attivita' fisiche **INTENSE** in **uno** di questi giorni?

_____ minuti

Attività moderate

2a Negli ultimi 7 giorni, per quanti giorni ha compiuto attivita' fisiche **MODERATE**, come ad esempio trasporto di pesi leggeri, giri in bicicletta ad una velocità regolare, attività in palestra, lavoro in giardino, lavoro fisico prolungato in casa… ? Non consideri le camminate

_____ giorni alla settimana

Nemmeno uno → (vada alla domanda 3a)

2b Quanto tempo in totale, normalmente, lei ha trascorso compiendo attivita' fisiche **MODERATE** in **uno** di questi giorni ?

_____ minuti

Cammino

3a Negli ultimi 7 giorni, per quanti giorni ha camminato per **almeno 10 minuti**?
(Consideri le camminate compiute al lavoro e a casa, quelle per spostarsi da un posto ad un altro ed ogni altra camminata che le e' capitato di fare per piacere, esercizio o sport)

_____ giorni alla settimana

Nemmeno uno → (vada alla domanda **4a**)

3b Per quanto tempo in totale, normalmente, lei ha camminato in **uno** di questi giorni?

_____ minuti

3c A che passo ha camminato prevalentemente?

passo **INTENSO**, che l'ha fatta respirare ad un ritmo molto più elevato del normale
passo **MODERATO**, che l'ha fatta respirare ad un ritmo solo moderatamente più elevato del normale
passo **LENTO**, senza alcun cambiamento nel suo ritmo di respiro

Attività da seduto

4a Negli ultimi 7 giorni, quanto tempo in totale lei ha trascorso rimanendo seduto, durante **un giorno** lavorativo?
(includa attività svolte al lavoro, a casa, mentre si recava la lavoro e durante il tempo libero: es. ad una scrivania, a tavola, mentre stava visitando degli amici, alla TV, leggendo)

_____ minuti

4b Negli ultimi 7 giorni, quanto tempo in totale ha trascorso rimanendo seduto, durante **un giorno** del fine settimana ?

_____ minuti

Interpretazione del questionario

Met attività intense = minuti * giorni * **8** Met	
Met attività moderate = minuti * giorni * **4** Met	
Met attività cammino = minuti * giorni * **3** se moderato, * **3,3** se intenso, * **2,5** se lento...	
Totale Met = Met att intense **+ Met** att moderate **+ Met** camminate = ...	

Se il totale è meno di **700** Met: SEI **INATTIVO**
Se il totale è tra 700 e 2519: SEI **SUFFICIENTEMENTE** ATTIVO
Se il totale è più di **2520** Met: SEI **ATTIVO O MOLTO ATTIVO**

11 Appendix C. General nutritional advices

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Consigli alimentari

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- Inizia la giornata facendo una **colazione completa**! Ci deve essere una fonte di proteine, una fonte di carboidrati e zuccheri semplici, grassi e fibra. Una colazione di questo tipo conserva il senso di sazietà più a lungo, regola il senso di fame durante la giornata e consente di arrivare al pasto successivo meno affamato. Per esempio: una tazza di latte oppure uno yogurt bianco, con dei biscotti secchi o delle fette biscottate o una manciata di cereali o del pane (meglio se integrali!), più qualche noce o mandorla è una colazione completa.
- "**IL PIATTO SANO**"

L'iconografia del "piatto sano" è un'immagine che rappresenta in modo semplice ed intuitivo come dovrebbe essere composto ogni pasto. A **pranzo** e a **cena** non fare mai mancare:

- 1 porzione di carboidrati (meglio se integrali)
- 1 porzione di proteine ("il secondo piatto")
- 1 contorno di verdure cotte o crude
- 1 frutto

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- Tra i carboidrati scegli tra pane, pasta, riso, orzo, farro, crackers, patate, ecc…
  - **N.B.** LE PATATE NON SONO VERDURA, sono una fonte di carboidrati. Attenzione all'associazione di più fonti di carboidrati nello stesso pasto (ad esempio pasta e pane, pane e patate, ecc..), perché si rischia di eccedere!
- Le fonti di proteine sono carne, pesce, formaggi, affettati, uova e legumi. Tra pranzi e cene ci sono 14 pasti, è importante variare la scelta dei secondi piatti. Le frequenze di consumo consigliate sono riportate nella tabella sottostante:

CARNE	3 vv/settimana (max 1 carne rossa)
PESCE	3-4 vv/settimana
FORMAGGI	2 vv/settimana
AFFETTATI	1-2 vv/settimana
UOVA	n. 2/settimana
LEGUMI	3 vv/settimana

- Evita il consumo di pesce crudo e prediligi i pesci con le branchie (orata, branzino, sardina, sgombro, salmone, ecc…), che apportano grassi buoni e poco colesterolo.
- I formaggi freschi (ricotta, primo sale, mozzarella, robiola) vanno preferiti a quelli stagionati, contengono meno grassi e meno sale. Attenzione alla quantità di grana sulla pasta/minestra/ecc..!!! Evita i formaggi erborinati.
- Tra gli affettati prediligi quelli magri come prosciutto cotto e fesa di tacchino, da consumare comunque con moderazione (porzione standard 50 g) perché ad elevato contenuto di sale. Evita insaccati, prosciutto crudo, speck, bresaola, ecc..
- Sia a pranzo che a cena fai spazio ad una bella porzione di **verdura di stagione**! Che sia cotta o cruda va bene sempre, aumenta il senso di sazietà a

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breve e lungo termine, inoltre la fibra facilita il transito intestinale e l'evacuazione. Ricordati di bere a sufficienza però, ogni giorno 1,5-2 L di acqua!

- Concludi il pasto con un **frutto di stagione**, senza esagerare (porzione standard 150-200 g)! La frutta è ricca di vitamine e minerali, ma anche di zuccheri che, se in eccesso, si depositano come trigliceridi nel tessuto adiposo. È consigliabile non superare le 2-3 porzioni di frutta al giorno, distribuendole al termine dei pasti principali, a colazione o negli spuntini!
    - Evita l'assunzione di pompelmo e melograno, in quanto interferiscono con i livelli plasmatici di Ciclosporina A e di Tacrolimus.
- Frutta e verdura vanno lavate bene, con l'utilizzo di amuchina o bicarbonato.
- Come spuntino a metà mattina/pomeriggio scegli uno yogurt bianco con un frutto tagliato dentro oppure un pacchetto di crackers o una manciata di frutta secca (mandorle/noci). Evitare snack come salatini, patatine, merendine, ecc..
- Evita o consuma saltuariamente le bevande zuccherate!
- Attenzione al consumo di alcol, le indicazioni sono: 1 unità alcolica al giorno per la donna, 2 per l'uomo!
- Preferisci come unico condimento l'olio extravergine di oliva (meglio se a crudo). Consuma con estrema moderazione burro, panna, margarine, salse, ...
- Riduci al minimo il consumo di sale, l'OMS ne raccomanda un consumo massimo di 5 g/giorno, corrispondenti a circa 2 g/giorno di sodio. Utilizza le spezie e le erbe aromatiche.
- La PIZZA è consigliabile non mangiarla più volte nell'arco della settimana. Quando la mangi cerca di non far mancare la porzione di verdura, un'ottima strategia può essere la pizza con le verdure.
- Impara a leggere le etichette dei prodotti confezionati, in questo modo potrai confrontare i prodotti in base al loro contenuto in grassi, zuccheri, fibra, sale, ecc..

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12 Appendix D. Food diary

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Diario alimentare e attivita' fisica

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REGIONE AUTONOMA FRIULI VENEZIA GIULIA

COGNOME E NOME: _____ GIORNO 1 (data): _____

PASTO	Alimenti e bevande consumati (specificare quantità)	Come mi sento dopo il pasto?	Attività fisica/ lavori domestici (tipo, intensità e durata)
Colazione h. _____			
Spuntino h. _____			
Pranzo h. _____			
Spuntino h. _____			
Cena h. _____			
Post-cena/ Bedtime h. _____			

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COGNOME E NOME: _____ GIORNO 2 (data): _____

PASTO	Alimenti e bevande consumati (specificare quantità)	Come mi sento dopo il pasto?	Attività fisica/ lavori domestici (tipo, intensità e durata)
Colazione h. _____			
Spuntino h. _____			
Pranzo h. _____			
Spuntino h. _____			
Cena h. _____			
Post-cena/ Bedtime h. _____			

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REGIONE AUTONOMA FRIULI VENEZIA GIULIA

COGNOME E NOME: _____ GIORNO 3 (data): _____

PASTO	Alimenti e bevande consumati (specificare quantità)	Come mi sento dopo il pasto?	Attività fisica/ lavori domestici (tipo, intensità e durata)
Colazione h. _____			
Spuntino h. _____			
Pranzo h. _____			
Spuntino h. _____			
Cena h. _____			
Post-cena/ Bedtime h. _____			

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COGNOME E NOME: _____ GIORNO 4 (data): _____

PASTO	Alimenti e bevande consumati (specificare quantità)	Come mi sento dopo il pasto?	Attività fisica/ lavori domestici (tipo, intensità e durata)
Colazione h. _____			
Spuntino h. _____			
Pranzo h. _____			
Spuntino h. _____			
Cena h. _____			
Post-cena/ Bedtime h. _____			

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NOTE: segnalare eventuali situazioni sociali o stati emotivi che possono avere interferito con la propria alimentazione (cercare di spiegare lo stato d'animo/emozione); eventuali disturbi fisici di gonfiore o di malessere dopo aver consumato certi alimenti (descrivere la sensazione e a che cibi la si correla);

_____
_____
_____
_____
_____
_____
_____

INFORMAZIONI PER IL PAZIENTE – COME COMPILARE IL DIARIO?

E' importante arrivare alla prima visita con il **diario alimentare** compilato e la **documentazione richiesta** (referti visite, esami del sangue, ecc..).

Il diario deve essere compilato per almeno 4 giorni, di cui 2 intrasettimanali e 2 weekend, e deve rispecchiare l'alimentazione abituale. Perciò, se sai già che in una determinata giornata hai una ricorrenza particolare e mangerai diversamente dal solito, tralascia quel giorno e riporta i successivi.

Non omettere cibi e bevande consumati durante queste giornate e non cambiare la tua alimentazione perché devi compilare il diario. Questo strumento è utile per capire al meglio le tue preferenze, i tuoi gusti e cercare di rendere il percorso nutrizionale più piacevole ed efficace possibile.

- Cerca di riportare **TUTTO quello che mangi e bevi** durante la giornata, anche gli alimenti che sembrano ininfluenti (zucchero, caramelle, cioccolatini, ecc);
- Sii il più preciso possibile, le quantità sono indispensabili ma va bene anche se usi le unità di misura casalinghe (1 cucchiaio piccolo/grande, 1 bicchiere, 1 tazza, ecc..) oppure il numero di pezzi (5 biscotti, 4 taralli, 3 fette biscottate);
- Riporta le modalità di cottura degli alimenti;
- Ricordati dei condimenti, specifica sempre quanti e quali condimenti utilizzi (2 cucchiai di olio/salsa, pizza con …(SPECIFICARE)..., 1 cucchiaio di parmigiano);
- Se usi prodotti confezionati, specifica la marca, il tipo e la quantità (es. colazione: 5 biscotti MACINE MULINO BIANCO);
- Ricordati anche le bevande che consumi: latte (scremato/pz scremato/intero oppure vegetale), succhi e spremute, i bicchieri di ACQUA/vino/birra/ecc..;

NB: E' consigliabile che la registrazione dei cibi e delle bevande assunte sia effettuata immediatamente dopo il loro consumo.

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13 Publications during candidature

- Lechiancole A, Vendramin I, Sponga S, Piani D, Benedetti G, Meneguzzi M, **Ferrara V**, Tullio A, Bortolotti U and Livi U. Bentall procedure with the CarboSealTM and CarboSeal ValsalvaTM composite conduits: long-term outcomes. Interact CardioVasc Thorac Surg 2021; Jun 28;33(1):93-100.

- Sponga S, Nagpal AD, Vendramin I, **Ferrara V**, Lechiancole A, Maiani M, Nalli C, Di Nora C, Guzzi G, De Manna ND, Bortolotti U and Livi U. Bridge to heart transplantation in patients with cardiogenic shock: a 20-year experience with two different surgical strategies. J Cardiovasc Med 2021, 22:388–395

- Lechiancole A, Vendramin I, Sponga S, Sappa R, Zanuttini D, Spedicato L, **Ferrara V**, Di Nora C and Livi U. Influence of donor-transmitted coronary artery disease on long-term outcomes after heart transplantation - a retrospective study. Transpl Int. 2020 Dec 1. Online ahead of print.

- Sponga S, Bonetti A, **Ferrara V**, Beltrami AP, Isola M, Vendramin I, Finato N, Ortolani F, Livi U. Preservation by cold storage vs ex vivo normothermic perfusion of marginal donor hearts: clinical, histopathologic, and ultrastructural features. J Heart Lung Transplant. 2020 Dec;39(12):1408-1416.

- Sponga S, Benedetti G, de Manna ND, **Ferrara V**, Vendramin I, Lechiancole A et al. Heart transplant outcomes in patients with mechanical circulatory support: cold storage versus normothermic perfusion organ preservation. Interact CardioVasc Thorac Surg 2020; doi:10.1093/icvts/ivaa280. Sept 2020 Online ahead of print.

- Lechiancole A, Vendramin I, Sponga S, Guzzi G, **Ferrara V**, Nalli C, Di Nora C, Bortolotti U, Livi U. Donor-recipient age interaction and the impact on clinical results after heart transplantation. Clin. Transplant, 2020

- Di Nora C, Sponga S, **Ferrara V**, Patriarca F, Fanin R, Nalli C, Lechiancole A, Vendramin I and Livi U. Emerging therapy in light-chain and acquired transthyretin-related amyloidosis: an Italian single-centre experience in heart transplantation. J Cardiovasc Med 2020, 21:000–000. Jul 2020 Online ahead of print.

- Cantarutti C, Fogolari F, Hunashal Y, **Ferrara V**, Caragnano A, et al. Assessing the Effect of Preservation in Heart Transplant Protocol: Cold Ischemia Versus Normothermic Perfusion. Biomark Applic, 2019; 03:139

- Gatti G, Sponga S, Peghin M, Givone F, **Ferrara V**, Benussi B, Mazzaro E, Perrotti A, Bassetti M, Luzzati R, Chocron S, Pappalardo A and Livi U. Risk scores and surgery for infective endocarditis: in search of a good predictive score, Scand Cardiovasc J 2019 Jun;53(3):117-124

- Lechiancole A, Sponga S, Vendramin I, Valdi G, **Ferrara V**, Nalli C, Tursi V and Livi U. Red blood distribution width and heart transplantation: any predictive role on patient outcome? J Cardiovasc Med (Hagerstown). 2019 Mar;20(3):145-151.

14 Contributions to national and international conferences

Oral presentations

- Trapianto di cuore e sindrome metabolica: un fattore di rischio sottostimato? **V. Ferrara***, S. Sponga, M. Marinoni, G. Valdi, C. Di Nora, C. Nalli, G. Benedetti, A. Lechiancole, M. Parpinel, U. Livi. 53° ANMCO National Congress, Rimini, Italy (19-21 May 2022)
- Metabolic Syndrome In Heart Transplantation: An Underestimated Risk Factor? **V. Ferrara***, S. Sponga, M. Marinoni, G. Valdi, C. Di Nora, C. Nalli, G. Benedetti, A. Lechiancole, M. Parpinel, U. Livi. 42nd Annual Meeting & Scientific Sessions of ISHLT, Boston, MA, USA (27-30 April 2022)
- Impact of autoimmune connective tissue diseases in cardiac surgery. Sponga S, **Ferrara V**, De Manna D, Dralov A, Dagenais F, Lechiancole A, Di Nora C, Vendramin I, Livi U, Voisine P. 30th SICCH Congress, Rome, Italy (16-18/12/2021)
- Trapianto di cuore e Sindrome Metabolica un fattore di rischio sottostimato? **V. Ferrara***, S. Sponga, C. Di Nora, C. Nalli, D. Piani, I. Vendramin, A. Lechiancole, U. Livi. 44th SITO, Naples, Italy (3/10/2021)
- Is heart transplantation a valuable option in patients with systemic rare diseases? Di Nora, C, **Ferrara, V**, Travaglini, C, Nalli, C, Lechiancole, A, Benedetti, G, Sponga, S, Vendramin, I, Livi. ESOT Congress, Milan, Italy (29-31/08/2021)
- Are heart transplant patients at higher risk for mortality following sars-cov-2 infection? Single centre experience. Sponga, S, Benedetti, G, **Ferrara, V**, Nalli, C, Di Nora, C, Lechiancole, A, De Manna, ND, Dralov, A, Vendramin, I, Livi. ESOT Congress, Milan, Italy (29-31/08/2021)
- Discarded grafts after cardiac ex-vivo preservation. Sponga, S, **Ferrara, V**, Beltrami, AP, Finato, N, Benedetti, G, Lechiancole, A, Nalli, C, Di Nora, C, Guzzi, G, Vendramin, I, Livi, U. ESOT Congress, Milan, Italy (29-31/08/2021)
- Distance between recipients residency and heart transplant center: effect on long-term outcome. Lechiancole, A, **Ferrara, V**, Sponga, S, Vendramin, I, Guzzi, G, Nalli, C, Di Nora, C, Piani, D, Livi, U. ESOT Congress, Milan, Italy (29-31/08/2021)
- Ex-vivo normothermic perfusion, a New Opportunity to Treat Complex cases. Sponga S, Ius F, Ferrara V, Royas S, Guzzi G, Lechiancole A, Sommer W, Kaufeld T, Haverich A, Livi U, Warnecke G. EACTS Congress, Barcelona, Spain (8-10/10/2020)

- Recipient or donor age in heart transplantation: which one fits more? C Di Nora, A Lechiancole, **V Ferrara**, S Sponga, V Tursi, G Guzzi, C Nalli, U Livi. ESC Congress, Paris, France (29/08 – 01/09/2020)
- Coronary Artery Disease of the Donor Graft: Any Impact on Survival and Cardiac Allograft Vasculopathy after Heart Transplantation? A Lechiancole, S Sponga, I Vendramin, **V Ferrara**, M Maiani, E Spagna, G Guzzi, C Nalli, M Meneguzzi, C Di Nora, D Piani, G Benedetti, V Tursi, D Zanuttini, U Livi. ISHLT Congress, Montreal, Canada (22-25/04/2020)
- Normothermic Ex-Vivo Perfusion for Donor Heart Preservation in Transplantation of Patients Bridged with Ventricular Assist Devices. S Sponga, F Ius, V Ferrara, S Royas, G Guzzi, A Lechiancole, W Sommer, T Kaufeld, A Haverich, U Livi, G Warnecke. ISHLT Congress, Montreal, Canada (22-25/04/2020)
- Aging with a new heart. Sponga, S, Di Nora, C, **Ferrara, V**, Lechiancole, A, Tursi, V, Nalli, C, Guzzi, G, Livi, U. ESOT Congress, Copenhagen, Denmark (15-18/09/2019)
- Heart transplantation in cardiac amyloidosis: an italian single centre experience. Di Nora, C, Sponga, S, Ferrara, V, Lechiancole, A, Tursi, V, Nalli, C, Livi, U. ESOT Congress, Copenhagen, Denmark (15-18/09/2019)
- Heart transplantation with donors age >= 60 years old: single centre experience. Di Nora, C, Sponga, S, Ferrara, V, Tursi, V, Guzzi, G, Nalli, C, Lechiancole, A, Livi, U. ESOT Congress, Copenhagen, Denmark (15-18/09/2019)
- Outcome of heart transplantation in short and long-term assistance device patients: cold storage vs normothermic perfusion. Sponga S, Di Nora C, **Ferrara V**, Tursi V, Lechiancole A, Vendramin I, Maiani M, Livi U. ESOT Congress, Copenhagen, Denmark (15-18/09/2019)
- Donor age in heart transplantation: results and controversies. C Di Nora, **V Ferrara**, S Sponga, A Lechiancole, V Tursi, C Nalli, G Guzzi, U Livi. ESC Congress, Paris, France (31/08-4/09 2019)
- Outcome of heart transplantation with marginal donors: cold storage vs. Normothermic perfusion. C Di Nora, S Sponga, **V Ferrara**, A Lechiancole, C Nalli, G Benedetti, A Beltrami, U Livi. ANMCO Congress, Rimini, Italy (16-18/05/2019)
- Ex-vivo Perfusion on Marginal Donors in Heart Transplantation: Clinical Resuts and Pathological Findings. Sponga, S, **Ferrara, V**, Beltrami, AP, Bonetti, A, Cantarutti, C, Caragnano, A, Ortolani, F, Lechiancole, A, Esposito, R, Di Nora, C, Tursi, V, Nalli, C, Livi, U. ISHLT Congress, Orlando, US (3-6/04/2019)

- Long-Term Survival after Heart Transplantation: Interaction between Donor and Recipient Age. A Lechiancole, S Sponga, **V Ferrara**, C Nalli, C Di Nora, G Guzzi, D Piani, M Meneguzzi, G Benedetti, V Tursi, U Livi. ISHLT Congress, Orlando, US (3-6/04/2019)
- Early and long-term results of late reoperation after repaired acute type a aortic dissection. D Piani, I Vendramin, A Lechiancole, **V Ferrara**, M Meneguzzi, S Sponga, U Livi. XXIX SICCH Congress, Rome, Italy (23-25/11/2018)
- Impact of a modified intraoperative setting for the management of circulatory arrest in type a acute aortic dissection. I Vendramin, D Piani, A Lechiancole**, V Ferrara**, M Meneguzzi, S Sponga, U Livi. XXIX SICCH Congress, Rome, Italy (23-25/11/2018)
- Outcome of heart transplantation with marginal donors: cold storage vs normothermic perfusion. Sponga S, **Ferrara V**, Beltrami AP, Bonetti A, Cantarutti C, Caragnano A, Esposito G, Lechiancole A, Guzzi G, Meneguzzi M, Nalon S, Ortolani F, Piani D, Livi U. XXIX SICCH Congress, Rome, Italy (23-25/11/2018)
- Assistenza ventricolare para-corporea nello scompenso cardiaco acuto: passato o presente? Sponga S, Di Nora C, **Ferrara V**, Ortis H, Benedett G, Maiani M, Tursi V, Nalli C, Vendramin I, Livi U. 42° SITO Congress, Bologna, Italy (22-24/11/2018)
- Trapianto cardiaco con donatori >60 anni di età: l'esperienza del nostro Centro. Di Nora C, Sponga S, Lechiancole A, Guzzi G, **Ferrara V**, Scoccimarro C, Tursi V, Nalli C, Livi U. 42° SITO Congress, Bologna, Italy (22-24/11/2018)
- Trapianto cardiaco con donatori marginali: trasporto ipotermico standard VS perfusione normotermica ex-vivo. Sponga S, **Ferrara V**, Di Nora C, Benedetti G, Lechiancole A, Nalon S, Nalli C, Beltrami AP, Caragnano A, Ortolani F, Bonetti A, Livi U. 42° SITO Congress, Bologna, Italy (22-24/11/2018)

Poster

- Mediterranean Diet and Metabolic Syndrome: a dietary intervention study to reduce metabolic syndrome risk after heart transplantation. **Ferrara V***, Marinoni M, Valdi G, Nalli C, Di Nora C, Sponga S, Benedetti G, Parpinel M, Livi U. XLII National Congress SINU, Naples, Italy (4-6 April 2022)
- Changes of body composition and dietary intakes after a nutritional intervention in a sample of heart-transplanted patients: primary longitudinal data. Marinoni M, **Ferrara V**, Valdi G, Nalli C, Di Nora C, Sponga S, Benedetti G, Parpinel M, Livi U. XLII National Congress SINU, Naples, Italy (4-6 April 2022)

- Adherence to the Mediterranean Diet in a sample of heart-transplanted patients from the University Hospital of Udine. Marinoni M, **Ferrara V**, Valdi G, Nalli C, Di Nora C, Sponga S, Benedetti G, Parpinel M, Livi U. XLII National Congress SINU, Naples, Italy (4-6 April 2022)
- Autoimmune Connective Tissue Diseases and Cardiac Surgery. Sponga S, **Ferrara V**, Dagenais F, de Manna ND, Dralov A, Lechiancole A, Vendramin I, Livi U, Voisine P. Circulation 2021; 144 (Suppl_1): A13880-A13880