



SAO PAULO STATE UNIVERSITY
“JÚLIO DE MESQUITA FILHO”
SCHOOL OF MEDICINE

Julí Thomaz de Souza

**Influence of protein and creatine
supplementation on functional capacity,
quality of life and muscle function
after stroke**

Thesis presented to the School of Medicine,
Sao Paulo State University “Júlio de Mesquita Filho”,
Campus Botucatu, to obtain the title of Ph.D. in
Pathophysiology in Internal Medicine

Advisor: Professor Paula Schmidt Azevedo Gaiolla

Co-advisor: Professor Rodrigo Bazan

Botucatu

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE JULÍ THOMAZ DE SOUZA, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM FISIOPATOLOGIA EM CLÍNICA MÉDICA, DA FACULDADE DE MEDICINA - CÂMPUS DE BOTUCATU.

Aos 05 dias do mês de maio do ano de 2023, às 10:00 horas, no(a) Sala da Congregação - FM/Botucatu - Unesp, realizou-se a defesa de TESE DE DOUTORADO de JULÍ THOMAZ DE SOUZA, intitulada **Influence of Protein and Creatine Supplementation on Functional Capacity, Quality of Life and Muscle Function After Stroke**. A Comissão Examinadora foi constituída pelos seguintes membros: Profa. Dra. PAULA SCHMIDT AZEVEDO GAIOLLA (Orientador(a) - Participação Presencial) do(a) Depto. de Clínica Médica / FM/Botucatu - Unesp, Prof. Dr. MARCOS FERREIRA MINICUCCI (Participação Presencial) do(a) Depto. de Clínica Médica / FM/Botucatu - Unesp, Prof. Dr. ADAM LEE GORDON (Participação Virtual) do(a) Medical School / University of Nottingham, Prof. Dr. GUSTAVO JOSÉ LUVIZUTTO (Participação Virtual) do(a) Depto. de Fisioterapia Aplicada / Universidade Federal do Triângulo Mineiro (UFTM), Prof. Dr. IVAN APRAHAMIAN (Participação Virtual) do(a) Faculdade de Medicina de Jundiaí (FMJ). Após a exposição pela doutoranda e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, a discente recebeu o conceito final: APROVADA. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Profa. Dra. PAULA SCHMIDT AZEVEDO GAIOLLA

Dedication

To my parents, Luiz and Fernanda, who always encouraged me and gave me all the support I needed to have the opportunity to follow my dreams.

To my sister Camila, who, in addition to being a sister, is my friend and has always been by my side in all important moments. To my nieces Serena and Videl, who are everything to me.

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Epigraph

“O real não está na saída nem na chegada: ele se dispõe
para a gente é no meio da travessia”

Grande sertão: Veredas - João Guimarães Rosa

“The truth is not in the setting out nor in the arriving: it comes to us
in the middle of the journey”

The Devil to Pay in the Backlands - João Guimarães Rosa

RESUMO

Introdução: O AVC é uma das principais causas de mortalidade e incapacidade. A fase aguda do AVC é marcada por inflamação, inapetência, disfagia, repouso no leito e alterações da mobilidade que podem comprometer o estado nutricional. Este estudo teve como objetivo avaliar o efeito da suplementação de creatina na massa muscular, força e capacidade funcional de idosos com AVC. Além disso, foram avaliados marcadores bioquímicos de inflamação, degradação muscular e síntese.

Metodologia: Ensaio clínico randomizado, duplo-cego, com idosos na fase aguda do AVC. Os participantes foram divididos em dois grupos: Tratamento (recebeu 10g de creatina 2x/dia) e Controle (recebeu 10g de placebo 2x/dia). Ambos os grupos receberam suplementação com proteína isolada do soro do leite em pó para atingir a meta de 1,5g de proteína/kg de peso corporal/dia e atendimento de fisioterapia diário. A intervenção ocorreu em 7 dias de internação. Verificou-se a influência da creatina na massa muscular (bioimpedância e ultrassonografia muscular), força (preensão manual e National Institutes of Health Stroke Scale), capacidade funcional (Escala de Rankin modificada), marcadores bioquímicos (3-Metil-histidina, Interleucina-6, Fator de crescimento semelhante à insulina tipo 1, fator de crescimento/diferenciação-15, procolágeno tipo III, insulina e progranulina). Acompanhamento para verificar capacidade funcional, força muscular, mortalidade e qualidade de vida 90 dias após o AVC.

Resultados: Trinta idosos foram incluídos em 2 grupos homogêneos, a maioria do sexo masculino com AVC isquêmico moderado. A suplementação de creatina não influenciou o peso corporal, força muscular, capacidade funcional e massa muscular avaliada por ultrassom e bioimpedância. No entanto, houve um aumento no índice de massa muscular apendicular em homens, mas não em mulheres. Não houve diferença estatisticamente significativa em relação ao uso de creatina e níveis séricos de IL-6 e IGF-1, bem como procolágeno Tipo III, insulina, GDF-15 e 3-Metil-histidina. A creatina influenciou na diminuição da progranulina. A oferta calórica de 21-25 kcal/kg com 1,5g de proteína/kg por dia mostrou-se adequada para a manutenção do peso independentemente da administração de creatina. A creatina não influenciou a mortalidade e a percepção de qualidade de vida 90 dias após o AVC.

Discussão: A suplementação foi segura com poucos eventos adversos relacionados e boa tolerância e aceitação. Houve um aumento no índice de massa muscular apendicular em

homens, mas não em mulheres. Esse aumento agudo da massa muscular pode ser devido ao edema muscular, uma vez que o transporte de creatina para dentro da célula depende do sódio. De fato, a resposta muscular à creatina é diferente em homens e mulheres. Observa-se que há potencial para a creatina modular alguns aspectos relacionados a biomarcadores inflamatórios no AVC agudo. A literatura mostra a associação entre aumento da progranulina e mortalidade e pior prognóstico funcional após o AVC. Em nossos achados, no grupo tratamento, observou-se redução da progranulina, mas não no grupo controle. Este resultado representa o papel anti-inflamatório da creatina. Assim, a novidade deste estudo é mostrar o potencial da creatina em modular a progranulina na fase aguda do AVC. O trabalho trouxe dados essenciais relacionados à ingestão calórico-proteica durante a internação, mostrando a importância da adequação nutricional para evitar a perda de peso nesse período.

Conclusão: A suplementação de creatina provou ser segura. Tem o potencial de modular alguns marcadores inflamatórios após o AVC e influenciou na redução dos valores de progranulina, refletindo seu papel anti-inflamatório. A creatina não afetou a capacidade funcional e a força, mas aumentou a massa muscular apendicular em homens. Estudos com maior número de participantes e maior tempo de intervenção devem ser realizados para melhor compreender a influência da suplementação de creatina na fase aguda do AVC.

Palavras-chave: Acidente vascular cerebral; Creatina; Músculo esquelético.

ABSTRACT

Background: Stroke is a leading cause of mortality and disability. The acute phase of stroke is marked by inflammation, loss of appetite, dysphagia, bed rest, and mobility changes that can compromise the nutritional status. This study aimed to evaluate the effect of creatine supplementation on muscle mass, strength, and functional capacity of older people with stroke. In addition, biochemical markers of inflammation, muscle degradation, and synthesis were assessed.

Methodology: Randomized, double-blind, clinical trial with older people in the acute phase of stroke. The participants were divided into two groups: Treatment (received 10g of creatine 2x/day) and Control (received 10g of placebo 2x/day). Both groups received supplementation with powdered milk protein serum isolate to achieve the goal of 1.5g of protein/kg of body weight/day and daily early mobility training. The intervention occurred in 7-days hospitalization. Were verified the influence of creatine on muscle mass (bioimpedance and muscle ultrasound), strength (handgrip and National Institutes of Health Stroke Scale), functional capacity (Modified Rankin Scale), biochemical markers (3-Methylhistidine, Interleukin-6, Insulin-like growth factor 1, Growth/Differentiation Factor-15, type III procollagen, insulin, and progranulin). Follow-up to verify functional capacity, muscle strength, mortality, and quality of life 90 days after stroke.

Results: Thirty older people were included in 2 homogeneous groups, mostly male with moderate ischemic stroke. Creatine supplementation did not influence body weight, muscle strength, functional capacity, and muscle mass evaluated by ultrasound and bioimpedance. However, there was an increase in appendicular muscle mass index in men but not in women. There was no statistically significant difference regarding the use of creatine and serum levels of IL-6 and IGF-1, as also Type III procollagen, insulin, GDF-15, and 3-Methylhistidine. Creatine influenced the decrease of progranulin. The caloric supply of 21-25 kcal/kg with 1.5g of protein/kg per day proved adequate for weight maintenance regardless of creatine administration. Creatine did not influence mortality and perception of quality of life 90 days after stroke.

Discussion: Supplementation was safe with few related adverse events and good tolerance and acceptance. There was an increase in appendicular muscle mass index in men but not in women. This acute increase in muscle mass may be due to muscle edema since creatine

transport into the cell depends on sodium. Indeed, the muscular response to creatine is different in men and women. It is observed that there is potential for creatine to modulate some aspects related to inflammatory biomarkers in acute stroke. Literature shows the association between increased progranulin and mortality and worse functional prognosis after stroke. In our findings, in the treatment group, the reduction in progranulin was observed, but not in the control group. This result represents the anti-inflammatory role of creatine. Thus, the novelty of this study is to show the potential of creatine to modulate progranulin in the acute phase of the stroke. The work brought essential data related to caloric-protein intake during hospitalization, showing the importance of nutritional adjustment to avoid weight loss during this period.

Conclusion: Creatine supplementation proved to be safe. It has the potential to modulate some inflammatory markers after stroke and influenced the reduction of progranulin values, reflecting its anti-inflammatory role. Creatine did not impact functional capacity, and strength but increased appendicular muscle mass in men. Studies with a larger number of participants and longer intervention time should be carried out to better understand the influence of creatine supplementation in the acute phase of stroke.

Keywords: Stroke; Creatine; Skeletal Muscle.

LIST OF ILUSTRATIONS

Figure 1	Flow diagram of the ICaRUS Stroke Trial	27
Figure 2	ICaRUS Stroke Trial template of recommended content for the schedule of screening, interventions, and assessments	32
Figure 3	Flowchart of inclusion of participants in the ICaRUS Stroke Trial from July/2019 to November/2022	46
Figure 4	Adverse events that occurred during participation of older people in the ICaRUS Stroke Trial	49
Figure 5	Body composition variables of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo	55
Figure 6	Appendicular muscle mass index of older during participation in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo corrected by sex	56
Figure 7	Muscle strength variables of older people during participation in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo	58
Figure 8	Functional capacity by Modified Rankin Scale of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo	59
Figure 9	Biochemical markers of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo	61
Figure 10	3MH biomarker by labeled isotope of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo corrected by sex	62
Figure 11	Kaplan-Meier survival curve of the older people during participation in the Icarus Stroke Trial	63

LIST OF TABLES

Table 1	Comparison between treatment and control groups of the general characteristics of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit	47
Table 2	Comparison between treatment and control groups of the neurological characteristics of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit	48
Table 3	Comparison between treatment and control groups of the body composition of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit	50
Table 4	Comparison between treatment and control groups of the muscle strength of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit	51
Table 5	Comparison between treatment and control groups of the biochemical exams and bio markers of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit	52
Table 6	Evaluation of the influence of creatine treatment on muscle mass in older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial	54
Table 7	Evaluation of the influence of creatine treatment on appendicular muscle mass index of older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial corrected by sex	56
Table 8	Evaluation of the influence of creatine treatment on muscle strength in older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial	57
Table 9	Evaluation of the influence of creatine treatment on functional capacity in older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial	59

Table 10	Evaluation of the influence of creatine treatment on biochemical markers of older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial	60
Table 11	Evaluation of the influence of creatine treatment on the 3MH biomarker by labeled isotope of older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial corrected by sex	62
Table 12	Comparison between treatment and control groups on the perception of quality of life, anxiety, and depression of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at the follow-up visit	64
Table 13	Comparison between treatment and control groups of results from 7-day food record of older people with ischemic stroke during the participation in the ICaRUS Stroke Trial	65
Table 14	Comparison between treatment and control groups of results from 24-hour Dietary Recall of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at the follow-up visit	65
Table 15	Consumption of food groups by older people with ischemic stroke who participated in the ICaRUS Stroke Trial	66

LIST OF ABBREVIATIONS AND ACRONYMS

AC: Arm circumference

AMA: Arm muscle area

AMMI: Appendicular muscle mass index

APMT: Adductor pollicis muscle thickness

ASPECTS: Alberta Stroke Program Early CT Score

BBM: Biceps brachii muscle

BMI: Body mass index

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

cm: Centimeter

ECW: Extracellular water

ELISA: Enzyme-Linked Immunosorbent Assay

ET: End of treatment

EuroQol-5D: The European Quality of life Scale Five Dimension

FAS: Full Analysis Set

FFMI: Fat free mass index

FFQ: Food Frequency Questionnaire

FOIS: Functional Oral Intake Scale

g: Grams

GDF-15: Growth/Differentiation Factor-15

h: Hour

HADS: Hospital Anxiety and Depression Scale

HbA1C: Hemoglobin A1C

HDL: High-density lipoprotein

HOMA-IR: Homeostasis assessment model of insulin resistance

ICaRUS: Influence of protein and Creatine supplementation on functional capacity, quality of life and mUScle function after Stroke

IGF-1: Insulin-like growth factor 1

IL-6: Interleukin-6

kg: Kilograms

kgf: Kilogram-force

kHz: Kilohertz

l: Liters

LACS: Lacunar syndrome

LDL: Low-density lipoprotein

m: Meter

3MH: 3-Methylhistidine

MHz: Megahertz

min: Minute

ml: Milliliter

mm: millimeter

MPB: Muscle protein breakdown

MPS: Muscle protein synthesis

mRs: Modified Rankin Scale

N: Number

NIHSS: National Institutes of Health Stroke Scale

NRS: Nutritional risk screening

P3NP: Type III procollagen N-terminal peptide

PACS: Partial anterior circulation syndrome

PL: Placebo

POCS: Posterior circulation syndrome

PP: Per-Protocol

RFM: Rectus femoris muscle

rpm: Revolutions per minute

SMM: Skeletal muscle mass

ST: Start of treatment

TACS: Total anterior circulation syndrome

TBW: Total body water

TNF- α : Tumour necrosis factor α

TOAST: Trial of Org 10172 in Acute Stroke Treatment

TR: Treatment

TST: Triceps skinfold thickness

US: Ultrasound

SUMMARY

1 INTRODUCTION	20
1.1 Stroke	20
1.2 Stroke and nutritional conditions	21
1.3 Biomarkers of inflammation, muscle breakdown and protein synthesis	22
1.4 Protein and creatine supplementation	23
2 HYPOTHESIS	25
3 AIMS	25
4 METHODOLOGY	26
4.1 Trial design	26
4.2 Participants and eligibility criteria	28
4.3 Interventions	28
4.4 Outcomes	30
4.5 Procedures	30
4.6 Randomization	33
4.7 Blinding	33
4.8 Security and data management	33
4.9 Protocol assessment	34
4.9.1 Clinical assessment	34
4.9.2 Nutritional assessment	35
4.9.3 Body composition assessment	36

4.9.4 Muscle strength assessment	37
4.9.5 Functional capacity assessment	38
4.9.6 Dependence degree assessment	38
4.9.7 Blood collection and biochemical dosages	38
4.9.8 Assessment of anxiety and depression	41
4.9.9 Quality of life assessment	41
4.9.10 Assessment of eating habits	41
4.10 Statistical analysis	42
4.11 Data monitoring	43
4.12 Ethics approval and consent to participate	44
5 RESULTS	45
6 DISCUSSION	67
7 CONCLUSION	74
REFERENCES	75
APPENDIX A - INFORMED CONSENT FORM	87
APPENDIX B - RESULTS OF MUSCLE STRENGHT AND FUNCTION AFTER 90 DAYS	90
ANNEX A - APPROVAL DOCUMENT OF RESEARCH ETHICS COMMITTEE FROM BOTUCATU MEDICAL SCHOOL	91
ANNEX B - APPROVAL DOCUMENT OF RESEARCH ETHICS COMMITTEE FROM BOTUCATU MEDICAL SCHOOL - AMENDMENT 1	95
ANNEX C - APPROVAL DOCUMENT OF RESEARCH ETHICS COMMITTEE FROM BOTUCATU MEDICAL SCHOOL - AMENDMENT 2	100

1 INTRODUCTION

1.1 STROKE

Stroke is a neurological syndrome with rapid development of clinical signs of focal disorders with symptoms lasting more than 24 hours. (1) It is an important public health issue, being the second leading cause of death in Brazil and worldwide. Stroke and cardiac ischemia were responsible for approximately 15 million deaths in 2015, equivalent to 85% of all deaths from cardiovascular diseases. (2)

Currently, around 3-4% of total health expenses in Western countries regard the monitoring, hospitalization and rehabilitation of stroke patients. In high-income countries, a decrease in the incidence of stroke and mortality has been observed in recent years, and these positive results may have occurred due to improved prevention with adequate control of risk factors, in addition to treatments in the acute phase of stroke and neurorehabilitation. However, stroke remains a major cause of disability worldwide. (3)

Ischemic stroke is characterized by sudden interruption of brain blood flow, caused by obstruction of a brain artery, occurring in around 80% of the cases and tending to have less serious consequences. The hemorrhagic stroke is characterized by the rupture of one of these arteries, promoting intracerebral blood leakage. (4)

Although stroke may happen at any stage of life and its risk factors are present in different age groups, it can be said that the older population is more vulnerable to the onset of chronic diseases, especially those of cardiovascular etiology. (5) According to the World Health Organization, it is estimated that in 2025 there will be more than 800 million older people in the world. The older population will increase mainly in Latin America and Asia, and in the next two decades the total number of stroke deaths will triple in these regions. (6,7)

Considering the importance of stroke, therapeutic modalities that aim to minimize the damage left by brain injury are being developed and improved. Prior to 1990, treatment options for stroke were limited and focused primarily on symptom management, secondary prevention, and rehabilitation. From 1995, there was a revolution in treatment with the approval of the Federal Drug Administration for the introduction of cerebral reperfusion therapy using intravenous tissue plasminogen activator, a treatment known as thrombolysis, which in recent years has been improved by more studies, as it had been the base of stroke treatment for approximately two decades. From 2015, more sophisticated clinical trials have

shown robust results for endovascular therapy, which has shown great efficiency in early arterial recanalization for patients with ischemic stroke. (8,9)

1.2 STROKE AND NUTRITIONAL CONDITIONS

Approximately two-thirds of individuals after a stroke has incomplete recovery, which makes this event one of the main causes of disability in adult life today. Patients normally follow an intense routine of interventions and treatments in the acute phase, subsequently moving on to rehabilitation, which aims to restore functions and/or minimize the sequelae left. (4) In this scenario, nutritional status deserves particular attention because it will directly influence the outcomes after stroke. Prior nutritional status has a direct influence in the outcomes after stroke. Indeed, people who were malnourished at the time of the stroke had more complications and higher mortality than those who were eutrophic, overweight, or obese. (10)

In addition, after stroke, the patient becomes more susceptible to involuntary weight, muscle and strength losses. The acute phase of stroke is marked by inflammation, loss of appetite, dysphagia, bed rest and mobility changes. Yet, according to the brain-affected area, muscle mass and function may be directly affected. The chronic phase of stroke is also associated with body composition alteration, which is proportional to the brain severity damage, dietary intake and chronic inflammatory aspects. (11)

Therefore, considering the current definitions of nutritionally related conditions, stroke patients are at risk of disease-related undernutrition with inflammation (cachexia) and stroke-related sarcopenia. (12,13) By definition, cachexia is a complex metabolic syndrome associated with underlying disease and characterized by muscle wasting with or without fat loss, in which weight reduction is an essential feature in adults, in addition to anorexia, inflammation, insulin resistance, and increased muscle protein breakdown. (13,14) A study that evaluated patients with mild and moderate stroke in the acute phase showed a 21% incidence of cachexia 12 months after the event. (15)

Regarding the definition of sarcopenia, it is considered a geriatric disease with a particular muscle decline related to ageing. However, the loss of muscle mass and function associated with an acute disease, like stroke, encompasses other mechanistic features related to metabolic stress. Its consequences are related to clinical outcomes such as hospitalization and length of hospital stay and other complications and mortality. (16)

The term Stroke-related sarcopenia would perhaps be substituted by "stroke-related muscle decline". Stroke-related muscle decline has peculiar characteristics, such as the rapid decline in muscle mass and changes in muscle structure. In addition, the brain injury's type, place, and size are essential in the differences in physical and functional performance because the most severe muscle mass loss occurs in the limb affected by the brain injury. (12)

Unlike age-related sarcopenia, after the stroke, muscle fibers change from slow to fast twitch fibers in the paretic limb. There is greater recruitment of type I fibers and less of type II fibers. Type I fibers present slow contraction and are more resistant to fatigue, while type II fibers have the opposite behavior. (12) A higher proportion of type II fibers was associated with lower gait speed in post-stroke patients. (17)

Indeed, after three weeks of stroke, the affected individuals significantly declined in muscle mass. After one year, there may be a loss of up to 3% of muscle mass in the paretic limb. This reduction is usually accompanied by fat deposition in the limb resulting in decreased strength and muscle function. Long-term disability is the most frequent complication after stroke, as 50% of patients suffer from hemiparesis, and 30% become unable to walk without assistance. (18–22)

1.3 BIOMARKERS OF INFLAMMATION, MUSCLE BREAKDOWN AND PROTEIN SYNTHESIS

Concerning the mechanisms that underlie the nutritional-related conditions after stroke, inflammation, immobilization, and low food intake are critical factors. They contribute to protein catabolism and lipolysis, which leads to insulin resistance, inhibiting muscle protein synthesis (MPS), and increased muscle protein breakdown (MPB). (23,24) Apoptosis, mitochondrial dysfunction has already been described as acute mechanisms involved in muscle mass and strength decline after stroke. (12) However, there is few data available about the potential biomarkers of inflammation, apoptosis, mitochondrial dysfunction, MPS and MPB in the acute phase of acute ischemic stroke.

Cardoso et al. published a review of the potential biomarkers for Frailty Syndrome. This review cited research in acute and chronic conditions with the potential association among inflammation [(Interleukin-6 (IL-6), progranulin), hormonal [(Insulin-like growth factor 1 (IGF-1)], neuronal (progranulin), apoptotic and mitochondrial dysfunction [(Growth/Differentiation Factor-15 (GDF-15)] and muscle decline. (25) However, the relationship between IL-6 with muscle health is contradictory. IL-6 is the primary chemokine involved in muscle regeneration.

Combined with IGF-1, IL-6 induces mTOR stimulus, insulin secretion, and muscle glucose uptake, contributing to MPS. Therefore, IL-6 and IGF-1 may indirectly reflect MPS. On the other hand, low-grade and chronic IL-6 increase is seen in ageing-related muscle disorders and reflects MPB. (26) Another compound, the type III procollagen, is associated with the anabolic response of the muscle and may also suggest the muscle response to a specific stimulus. (27)

The role of Progranulin is also controversial because some studies in chronic conditions, such as dementia and mainly in experimental studies, evidenced this biomarker's anti-inflammatory and protective effect. However, the progranulin level was related to inflammation and poor prognostic in humans and acute conditions such as stroke and myocardial infarction. (28,29)

GDF-15 mediates acute stress signals and is a marker for mitochondrial disease and apoptosis. (25) The 3-methylhistidine is a marker of muscle degradation. So, the two biomarkers could suggest the presence of MPB.

Therefore, the behaviour of muscle mass and function and the role of biomarkers in the acute phase of stroke remain to be elucidated. Taken together, few studies investigated the role of nutritional intervention in the acute phase of stroke and their potential of mitigating the muscle decline.

1.4 PROTEIN AND CREATINE SUPPLEMENTATION

Post-stroke nutrition guidelines do not provide specific recommendations on caloric and macronutrient intake in this population. For older people, there is a recommendation for intake of 1.2 to 1.5 g of protein per kg of body weight per day, associated with physical activity to prevent sarcopenia, and this need for a high-protein diet occurs because older individuals are more susceptible to inadequate intake of this nutrient, which can happen due to several factors related to age itself, such as reduced appetite, gastrointestinal problems, reduced energy needs, changes in food preference, anabolic resistance, insulin resistance and association with chronic and inflammatory diseases that can lead to increased proteolysis. (30,31)

Some nutrients, such as creatine, have their role studied in hypertrophy and improvement of muscle function. Supplementation with creatine is effective in improving muscle mass in healthy older people and pathological conditions, with positive results

supported by studies in different populations. The effects of this nutrient supplementation on brain metabolism are quite promising. (32)

Creatine (methyl guanidine-acetic acid) is a compound formed from the junction of the amino acids arginine, glycine, and methionine and is synthesized in the liver, kidneys, and acinar cells of the pancreas. In addition to endogenous synthesis, creatine can be ingested through the consumption of animal foods or supplementation. (33) Most of the creatine in the human body is in skeletal muscle in its phosphorylated form and acts in the formation of adenosine triphosphate in high-intensity, short-duration exercises that use fast-twitch muscle fibers. This compound has the potential to neutralize some morphological and metabolic parameters that sustain sarcopenia through direct and indirect mechanisms, including roles in bioenergetics, anabolic and anti-catabolic functions, and increased muscle regeneration capacity by facilitating the availability of muscle stem cells. In the body, creatine is converted to creatinine by a dehydration reaction to be eliminated in the urine. (34)

Cocktail supplementation of compounds involving isolated whey protein, omega 3, vitamin D, calcium, and creatine along with physical activity allowed healthy subjects to preserve muscle mass and function for 1 year. (35)

Another study developed with healthy older people showed that creatine supplementation versus placebo associated with resistance exercise resulted in an increase in lean mass after 12 weeks of intervention. Positive results of protein and creatine supplementation in improving muscle mass can be found in other populations such as older women and healthy adults.(36,37) However, the action of specific nutrient supplementation in individuals who have suffered a stroke is still little known, and studies in this population are few.

Concerning physical exercise, is fundamental for the maintenance and recovery of muscle mass and function in any clinical situation, in addition to promoting an important role in the quality of life, and the recovery of independence, and may mitigate the damage caused to muscle tissue. (35)

Considering the changes that occur in the body after the stroke, it can be said that it is extremely important that these individuals receive adequate rehabilitation treatment after the event, especially physical therapy. A recent study shows that physiotherapy sessions should be started even during hospitalization, as soon as possible, and increasingly affirms the

importance of following a physiotherapy care protocol to promote functional improvement in patients affected by stroke. (38)

In summary, advanced age, immobilization, inflammation, hormonal changes, and neurodegeneration are factors that can potentially increase the risk of sarcopenia after stroke.

Although it is known that older people have a greater need for protein to compensate for anabolic resistance and hypermetabolic diseases and that the combination of resistance exercise and an adequate protein diet is recommended to maintain healthy skeletal muscles, there is still no recommendation for this type of multimodal therapy for individuals after stroke. (39) Additionally, little is known about the influence of protein supplementation and specific amino acids associated with physical activity on the functional capacity and quality of life of this population.

2 HYPOTHESIS

Creatine supplementation associated with early mobility training improves muscle mass, strength, and function after stroke and modulate the metabolic response to the acute stress.

3 AIMS

Primary

To verify the influence of creatine supplementation on changes in muscle mass, muscle strength, and functional capacity during hospitalization for stroke.

Secondary

To describe and compare potential serum biomarkers of inflammation, hormonal status, mitochondrial and apoptotic activities, MPS and MPB between the group that received creatine supplementation and control group.

To verify functional capacity, muscle strength, mortality, and quality of life in a follow-up 90 days after the stroke between the group that received creatine supplementation and control group.

To assess the nutritional intake pattern and safety related to energy, protein and creatine supplementation during hospitalization.

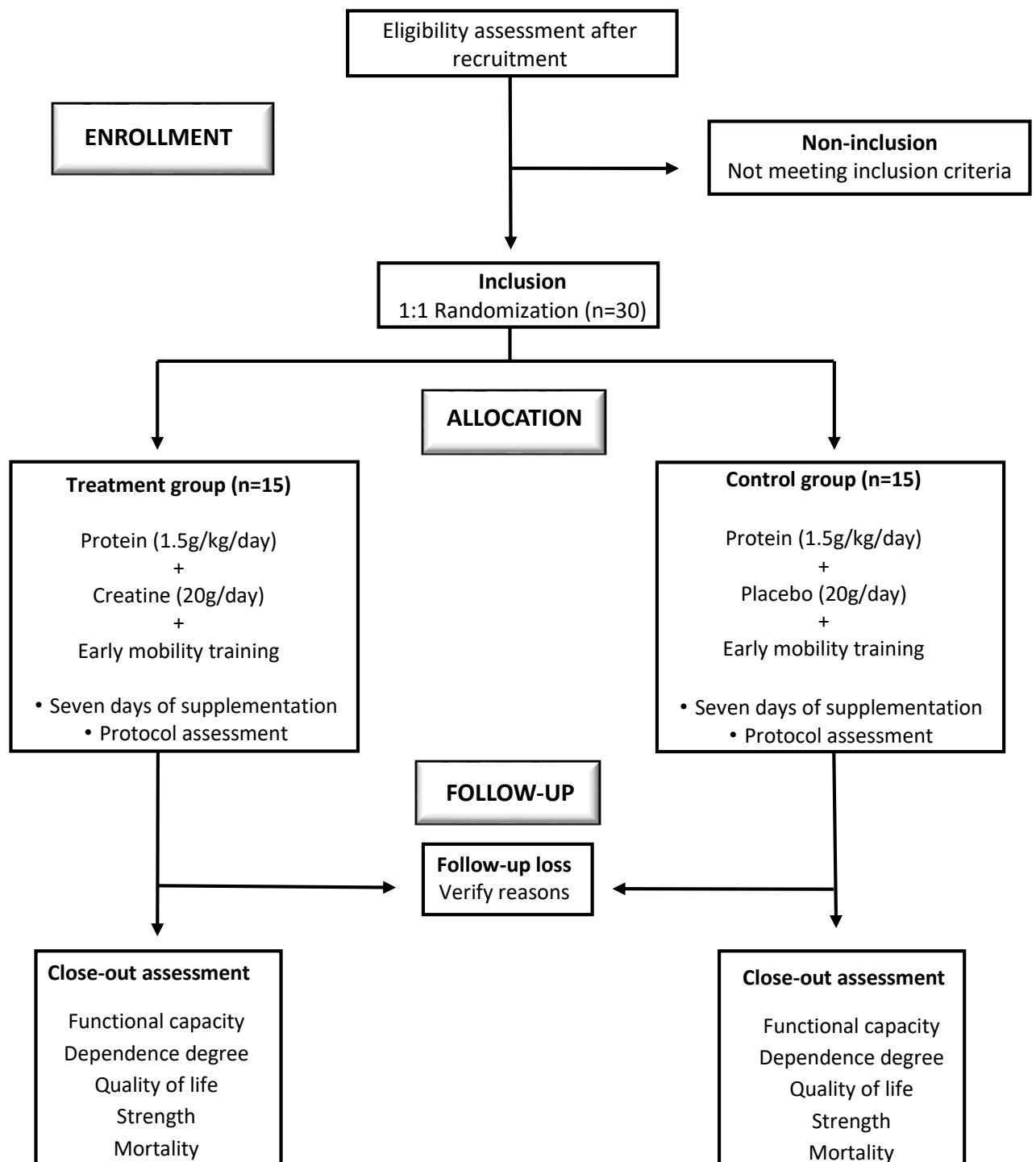
4 METHODOLOGY

4.1 TRIAL DESIGN

This is a randomized, double-blind, unicenter, parallel-group trial including individuals in the acute phase of ischemic stroke to verify the superiority of the treatment compared to the control group.

The acronym ICaRUS Stroke Trial was chosen for the study, which comes from the title “Influence of protein and Creatine supplementation on functional capacity, quality of life and mUScle function after Stroke”

The duration of the trial for the individual subject was approximately 90 days, and each subject attended a maximum of three visits (First 24h after stroke, 7th day of hospitalization, and 90 days after stroke). A flow diagram of the study is presented in Figure 1.

Figure 1 - Flow diagram of the ICaRUS Stroke Trial

4.2 PARTICIPANTS AND ELIGIBILITY CRITERIA

Men and women aged 60 years and older who were diagnosed with ischemic stroke, without previous disability (Modified Rankin Scale [mRs] – [mRs≤2]) and agree to sign the informed consent form were recruited in the first 24 hours after stroke.

Non-inclusion criteria - Participants with hemodynamic instability, mechanical ventilation, pacemakers and metal prostheses due to interference in bioimpedance results, previous kidney disease, creatinine clearance ≤ 30 ml/min/1.73m², history of malabsorptive gastrointestinal surgery, parenteral nutrition, allergies or intolerance to any component of the study products, and individuals who decline to participate.

Exclusion criteria - Participants who were intolerant of supplements, who were unable to stay in the stroke unit or who withdrew their consent.

The study was conducted at the Stroke Unit of the Clinical Hospital of Botucatu Medical School, Brazil, and the follow-up visits at the Neurovascular Disease Outpatient Clinic of the same institution. Recruitment was carried out by daily monitoring of admissions to the Stroke Unit. Possible candidates were screened and invited to participate in the study.

Sample Size

We reviewed a study that analyzed the effects of creatine supplementation in 18 healthy older people, in which 10 patients were allocated to the creatine group and 8 to the control group. The authors observed an improvement in muscle strength, weight, and fat-free mass in the group that received supplementation. (40) In addition, a meta-analysis based on this previous study included approximately 600 patients from 60 studies, with an average of 10 patients in each arm per study. (41) Considering that this is a study on creatine supplementation in patients after stroke, we decided to include 50% more in the convenience sample, with a total of 15 patients in each group.

4.3 INTERVENTIONS

Creatine supplementation was administered in the treatment group to avoid muscle loss which is common in patients after stroke. The control group received maltodextrin, a complex carbohydrate with no direct effect on muscle loss. All participants received the same

amount of protein per kg of body weight so that the groups were homogeneous in the consumption of this nutrient. The intervention was carried out as follows:

Treatment group (15 participants) - One sachet containing 10 g of creatine was administered twice a day.

Control group (15 participants) - One sachet containing 10 g of placebo (maltodextrin) was administered twice a day.

All sachets were the same color and size. All patients received assisted supplementation with powdered milk protein serum isolate, when necessary, to achieve a daily intake of 1.5 g/kg of body weight and early mobility training with progressive dose performed by trained physiotherapists started after 24 hours. Supplementation was offered during the 7-day hospitalization. The supplements were diluted in 100 ml of water at room temperature.

Rehabilitation started after the first 24 h of the ictus, and the intensity was proportional to the benefit and tolerance of each individual. Early mobility training was performed according to the current rehabilitation guidelines for stroke patients and consists of active sitting, standing and walking exercises associated with task-oriented training for 20 minutes, 2 times a day, with light to moderate intensity. (42,43)

The protocol followed was applied by the team of physiotherapists specialized in stroke at the Rehabilitation and Physiotherapy Unit of the Clinical Hospital of Botucatu Medical School, Brazil, and was performed in the routine of patients during hospitalization in the Stroke Unit.

All individuals were instructed at the time of hospital discharge on healthy eating habits, and adequate fractioning, and provided incentives for eating fruits, vegetables, and whole grains and maintaining water intake. Proper food preparation was encouraged, avoiding frying by immersion in oil, and the consumption of processed foods was discouraged. All orientations were under the Dietary Guidelines for the Brazilian Population by the Ministry of Health. (44)

Participants who were intolerant of supplements were excluded from the study. All participants were followed up daily and were encouraged to consume all meals and study products. When necessary, substitutions were made to the patient's diet with similar foods to increase food and supplementation acceptance during the intervention period. Empty sachets were kept by the research team for checking daily.

4.4 OUTCOMES

Primary outcomes

The primary outcomes were changes in muscle mass, muscle strength, and functional capacity after the intervention. The evaluations were conducted in the first 24 hours after the stroke and on the 7th day of hospitalization.

The participants were assessed for the following:

- 1) Muscle mass by muscle ultrasonography, and electrical bioimpedance.
- 2) Muscle strength by handgrip and lower limb strength according to the National Institutes of Health Stroke Scale (NIHSS) item 6 score.
- 3) Functional capacity according to the mRs.

Secondary outcomes

- 1) Potential serum biomarkers of inflammation, hormonal status, mitochondrial and apoptotic activities, MPS and MPB assessed by analysis of IL-6, progranulin, IGF-1, type III procollagen, insulin and 3-methylhistidine (3MH) values performed at the inclusion visit and after 7 days of intervention.
- 2) Functional capacity assessed by mRs and Timed Up and Go test; muscle strength assessed by handgrip, lower limb strength according to the NIHSS item 6 score, and 30-second Chair Stand test; mortality; and quality of life assessed by The European Quality of life Scale Five Dimension (EuroQol-5D) in the follow-up visit.
- 3) Nutritional intake pattern and safety related to energy, protein, and creatine supplementation assessed by Food record of the 7 days of intervention, and 24-hour Dietary Recall and Food Frequency Questionnaire in the follow-up visit.

4.5 PROCEDURES

All patients with ischemic stroke confirmed by imaging examinations who meet the study inclusion criteria were invited to participate. After signing the informed consent form, the individuals were randomized to the treatment or control groups. All participants were submitted to clinical and neurological evaluations conducted by the medical team using the NIHSS score, which is used to determine treatment and predict prognosis; Bamford's clinical

classification according to brain injury location; and the Alberta Stroke Program Early CT Score (ASPECTS), which verifies the extent of the injury. (1,45,46)

The template for the recommended schedule of enrollment, interventions, and assessments is presented in Figure 2. To verify the occurrence of mortality, we access death certificates from medical records

Figure 2 - ICaRUS Stroke Trial template of recommended content for the schedule of screening, interventions, and assessments

PROTOCOL ACTIVITY	Screening	Randomization	Treatment phase		Follow-up
			ST	ET	
VISIT			Day 1	Day 7	Day 90
Eligibility screening	x				
Informed consent	x				
Clinical evaluation ^(a)	x				
Allocation		x			
INTERVENTIONS					
Treatment group			x	x	
Control group			x	x	
ASSESSMENTS					
Nutritional Assessment ^(b)			x	x	
NRS 2002			x		
Multifrequency electrical bioimpedance			x	x	
Muscle ultrasound			x	x	
D3-Methylhistidine			x	x	
NIHSS			x	x	x
Handgrip			x	x	x
30-second Chair Stand test				x	x
Barthel Index				x	x
Timed Up and Go test				x	x
Modified Rankin Scale			x	x	x
Biochemical dosages			x	x	
Food frequency questionnaire					x
24-hour Dietary Recall					x
EuroQol-5D					x
HADS					x
Mortality					x

ST: Start of treatment; **ET:** End of treatment; **(a):** Anamnesis, past medical history, National Institute of Health Stroke Scale (NIHSS), Bamford classification, and Alberta Stroke Program Early CT Score (ASPECTS); **(b):** Weight, height and body mass index; **NRS 2002:** Nutritional risk screening; **EuroQol-5D:** The European 5D Quality of life Scale; **HADS:** Hospital Anxiety and Depression Scale.

4.6 RANDOMIZATION

For all subjects, the screening visit and the enrollment visit were performed on the same day. Subjects were randomized 1:1 to the treatment or control groups. The randomization was performed using a cell phone application (Randomizer), which creates a random control and treatment sequence and was performed every 10 out of 10 participants. No stratification factor was used.

4.7 BLINDING

To maintain the blindness of the study, randomization was performed by a person uninvolved in data collection and data analysis. That same person separated the creatine or placebo sachets into numbered and sealed envelopes and handed them to the researcher responsible for administering the supplements to the participants.

The participant, the professional responsible for administering the supplements, the professionals responsible for the biochemical analyzes and the person responsible for conducting the protocol assessments were blinded to the intervention. The professional in charge of the statistical analysis of the data was not blinded to the intervention.

Breaking the blinding would have been allowed if the participant had a serious adverse effect related to the supplementation, such as an allergic reaction. In this case, the principal investigator would be responsible for breaking the blinding and for informing the hospital's medical staff about which supplement the participant was consuming.

4.8 SECURITY AND DATA MANAGEMENT

All collected data was de-identified and stored in a safe place to protect the confidentiality of each participant. The data will be stored for 5 years after the the end of the research and after that period it will be destroyed.

Subjects in both groups were evaluated in the first 24 h after the stroke, after 7-day hospitalization, and 90 days after stroke. All individuals who participate in the study were instructed to complete the protocol during the 7 days of hospitalization in the stroke unit.

The research team carried out daily visits during the 7 days of hospitalization to guarantee the participant's permanence in the study. After hospital discharge, the team contacted the participant by telephone to ensure the follow-up visit 90 days after the stroke.

All data was double-checked by 2 team researchers to promote data quality (e.g., double data entry; range checks for data values).

4.9 PROTOCOL ASSESSMENTS

4.9.1 CLINICAL ASSESSMENT

The clinical evaluation for the diagnosis of the stroke, its complications, and its severity was performed by the medical team upon the patient's arrival at the hospital. Data related to anamnesis, previous clinical history, and relevant information related to the patient were verified in the electronic medical record and recorded in the study protocol. For the diagnosis of stroke, in addition to carrying out biochemical and imaging tests, such as computed tomography and/or magnetic resonance imaging, concomitantly, predictive scales for the prognosis and severity of the disease should be applied, such as the NIHSS scale, which it is currently used to determine the most appropriate treatment and to predict the patient's prognosis, with reproducibility, safety, and validity in this population. It has 11 categories with 15 items of neurological exams to assess the effect of stroke and its possible total score is from 0 to 42, with higher scores indicating greater deficits. (45)

In addition to the NIHSS, other scales are used to assess stroke. Bamford's clinical classification, which is divided into: Lacunar syndrome (LACS), Total anterior circulation syndrome (TACS), Partial anterior circulation syndrome (PACS), and Posterior circulation syndrome (POCS) was applied. (1) Another classification used was Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, which is divided into five categories: Large-vessel atherothrombosis, Cardioembolism, Small-vessel disease, Other determined causes, and Undetermined causes. (47)

The ASPECTS score, which verifies the extent of the injury was applied. The ASPECTS score evaluates 10 areas in 2 axial slices on the cranial tomography. Each area corresponds to 1 point, and the score ranges from 0 to 10, with 10 being the "normal" score. (46)

The Functional Oral Intake Scale (FOIS) was applied to assess changes in diet consistency, which may indicate the presence of dysphagia. The scale comprises seven levels ranging from 1 to 7 and is designed to grade oral intake. The levels are Level 1: Nothing by mouth; Level 2: Tube dependent with minimal attempts of food or liquid; Level 3: Tube dependent with consistent oral intake of food or liquid; Level 4: Total oral diet of a single consistency; Level 5: Total oral diet of multiple consistencies but requiring special preparation or compensations; Level 6: Total oral diet of multiple consistencies without special preparation but with specific food limitations, and Level 7: Total oral intake without restrictions. (48)

4.9.2 NUTRITIONAL ASSESSMENT

All individuals were submitted to nutritional assessment by the same dietitian and the following items were evaluated:

Weight was measured with the patient barefoot, wearing only hospital pajamas on a weight scale (Relaxmedic Your Way), showing body weight in kg. Height was measured using a portable ultrasonic stadiometer (InLab-InBody) with the patient barefoot and in an orthostatic position, showing height in meters. The body mass index (BMI) was calculated according to the Quetelet equation ($\text{BMI (kg/m}^2\text{)} = \text{weight/height}^2$). (49)

The thickness of the rectus femoris and biceps brachii muscles, both affected and unaffected by the stroke, was measured with a BodyMetrix BX-2000 A mode ultrasound device (Intelametrix, Livermore, CA, USA) with a 2.5-MHz transducer. Measurements were performed on both sides of the body, in a supine position, with the application of transmission gel at the measurement sites and soft application of the transducer perpendicularly. Each location was measured two or three times, based on the software's compliance with these measurements, and the average was used to represent the final thickness. (50)

The Nutritional Risk Screening 2002 (NRS 2002) was applied to all participants at the baseline visit. This tool considers anthropometric variables, food intake, disease severity, and patient age to assess the presence of undernutrition and the risk of developing undernutrition during hospitalization. A score of $\text{NRS} \geq 3$ indicates nutritional risk. (51)

4.9.3 BODY COMPOSITION ASSESSMENT

Multifrequency electrical bioimpedance (Seca model mBCA 525, Hamburgo, Germany), which is an equipment composed by the primary monitor and a rubber belt linked to 4 lines was performed with all participants at the inclusion visit and on the seventh day of hospitalization.

Electrical bioimpedance measures the electrical resistance and reactance by the passage of low-voltage, and high-frequency electrical current through tissues. The impedance is expressed in ohms and measures the resistance to the passage of electric current, and is inversely related to electrical conductivity. Resistance is also expressed in ohms and measures the opposition offered by the body to a low electrical current. Resistance is inversely related to the water and electrolyte content of the tissues. Reactance is expressed in ohms, and is related to the capacitance properties of cell membranes. Variations may occur depending on their integrity, function and composition. The phase angle is expressed in degrees and reflects the relative contribution of body fluids and cell membranes in the human body. It is positively associated with capacitance and negatively associated with resistance. (52)

The evaluations were performed in the morning. Participants were asked to: do at least 4 hours of fasting, empty the vesical bladder, remove metal pieces of the body and lay down in the supine position, with legs apart, and hands open downwards and away from the body. Then, pairs of disposable electrodes (3M™ Red Dot™) were placed on the dorsal surface of both hands and feet. Finally, the rubber belt was placed on the patient's thigh and the lines were attached to the electrodes to assess the impedance of the trunk, arms, and legs at nine different current frequencies (1 kHz, 2 kHz, 5 kHz, 10 kHz, 20 kHz, 50 kHz, 100 kHz, 200 kHz and 500 kHz) providing the impedance of the whole body and segments. Phase angle (degrees) was measured at 50 kHz; using the formula $\text{PhA}(\text{degrees}) = \arctan(X_c/R) \times (180/\pi)$. Impedance measures were also included to estimate segmentary and total fat-free mass, fat mass, muscle mass, total body water, and extra and intracellular water from the whole body or in separate parts.

The appendicular muscle mass index (AMMI) was calculated using data obtained from electrical bioimpedance, adding the skeletal muscle mass values of the four limbs and dividing by squared height. The result was expressed in kg/m^2 . (53)

4.9.4 MUSCLE STRENGTH ASSESSMENT

Handgrip was measured using a manual hydraulic dynamometer (Saehan Hydraulic Hand Dynamometer®, Model SH5001, Saehan Corporation, Korea), which measures the amount of force produced by an isometric contraction applied to the handles, and handgrip was recorded in kilogram-force (kgf). The participants were seated, with the shoulder slightly adducted, the elbow flexed at 90°, the forearm in a neutral position with the wrist position varying from 0° to 30° of extension. Three measurements were performed and the maximal value achieved was recorded with a 15-second rest interval between measurements on the affected and unaffected sides. (54) The handgrip cutoff points were <27 kg for men and <16 kg for women. (55)

Lower limb strength was assessed according to the 30-second Chair Stand test using a chair placed against a wall. The test began with the patient sitting in the chair, with their back straight, feet aligned with the shoulders, and the arms crossed at the wrists and held against the chest. A chair with no arms and rubber ends on the legs was used. The chair was placed against a wall to prevent movement during the test. The test began with the patient sitting in the middle of the chair, back straight, feet shoulder-width apart, and placed on the floor at a slight angle to the back of the knees, with one foot slightly in front of the other to help maintain balance when standing foot. The arms were crossed at the wrists and held against the chest. At the “go” signal, the patient stood up straight and returned to the initial sitting position. Subjects were encouraged to complete as many cycles as possible within a 30-second time limit. The number of correctly executed cycles within 30 seconds was counted, and the incorrect cycles were discarded. (56) The cutoff point for this assessment was >15s to complete 5 cycles. (55)

The NIHSS item 6 score was used to verify lower limb strength. The participant was placed in a supine position with the evaluated leg extended to 30°, and we evaluated whether the limb falls within 5 seconds. Each limb is tested in turn, beginning with the nonparetic leg. This item is scored as follows: 0 = no drift, the leg is held in the 30-degree position for the full 5 seconds; 1 = drift, the leg falls before the end of the 5 seconds but does not touch the bed; 2 = some effort against gravity, the leg falls to the bed before 5 seconds although there is some effort made against gravity; 3 = no effort against gravity, the

leg falls to the bed immediately; 4 = no movement; and UN = untestable owing to amputation or joint fusion at the hip. (57)

4.9.5 FUNCTIONAL CAPACITY ASSESSMENT

Developed in 1988, mRs is one of the most used tools to assess functional outcomes after stroke and its score ranges from 0 to 6. Patients grouped as scores 0 and 1 are classified as independent, they did not have any sequelae or these were considered minimal; Patients classified as 2 and 3 are those who have some sequelae, but through adaptations, they can do the previous activities and walk without help; Patients classified as 4 and 5 are unable to walk without assistance and may be confined to bed, requiring a 24-hour caregiver; Death is classified as score 6. (45) Participants with mRs $\geq$ 3 score was considered with disability.

The Timed Up and Go test is a commonly used screening tool for fall risks. Its main purpose is to assess mobility and balance. It was developed in 1991 and to carry out the test, the participant had their time measured while they got up from a chair, walked in a straight line 3 meters away (at a comfortable and safe pace), turned around, walked back and sat down up on the chair again. (58) The cutoff point for this assessment was $\geq$ 20s for both sexes. (55)

4.9.6 DEPENDENCE DEGREE ASSESSMENT

The Barthel Index was used to measure functional independence and mobility in chronically ill patients. The index can be completed through direct observation, clinical records, or applied by the patient himself. It intends to verify if the individual can perform certain tasks independently. The scale consists of 15 items, with scores ranging from 0 to 100 (0–20 indicating total dependence; 21–60 severe dependence; 61–90 moderate dependence; 91–99 light dependence, and 100 independence). (45)

4.9.7 BLOOD COLLECTION AND BIOCHEMICAL DOSAGES

The biochemical tests performed in the routine of hospitalization for stroke were noted in the study protocol. During the first 24 hours of hospitalization, renal function was

evaluated with creatinine and urea tests, and creatinine clearance was calculated using the CKD-EPI equation.

For the biochemical dosages, 12 ml of blood was collected per participant, in 3 dry tubes with a capacity of 4 ml each, centrifuged at 3500 rpm for 15 minutes, the serum was separated and divided into 5 identified aliquots and stored in specific boxes in a freezer at -80°C. Blood collection was performed in the first 24 hours after the stroke and on the 7th day of hospitalization.

Analysis of insulin, IGF-1, interleukin-6, progranulin, type III collagen, GDF-15, and 3MH levels was performed by Enzyme-Linked Immunosorbent Assay (ELISA), in which the amount of protein in the extract will be determined using the Bradford method with the final concentration adjusted to 1 mg/ml.

Insulin is a hormone with an anti-catabolic effect acting as a protein synthesis stimulator. It is a pleiotropic hormone with several functions in the body maintaining blood glucose levels and acting in cell proliferation. (59) For insulin evaluation, the E-EL-H2665 kit was used - range 0.78-50 μ IU/mL - with a dilution of 1 to 5.

IGF- is a hormone with multiple functions in the body, it is involved in cell proliferation, the inflammatory process, the metabolism of lipids and proteins, frailty, and aging. (25) In stroke, reduced levels of IGF-1 correlate with increased mortality rate, worse functional outcomes, and increased morbidities after stroke. (60) For IGF-1 evaluation, the E-EL-H0086 kit was used - range 1.56-100 ng/mL - with a dilution of 1 to 5.

Interleukin-6 induces metabolic changes usually found in catabolic states, increasing plasma glucose concentrations without significantly altering plasma insulin concentrations. It is an inflammatory cytokine that is associated with severity and worse functional prognosis after stroke. (61) Under conditions of physical exercise, it is considered a myokine and can be produced by skeletal muscle acting on adipose tissue promoting lipolysis and reducing inflammation. (62,63) For IL-6 evaluation, the E-OSEL-H0001kit was used - range 3.13-200 pg/mL - with a dilution of 1 to 2.

Progranulin is a growth factor that is expressed in many tissues (epithelia, bone marrow, immune system cells, and organs) and has been considered to be an anti-inflammatory protein. Progranulin loss has been observed after acute brain injury, contributing to an increase in the expression of pro-inflammatory genes, excessive microglia activation, and to autophagy impairment. Nonetheless, plasma and cerebrospinal fluid levels

of progranulin are thought to increase during healthy aging. Anyway, progranulin can be a good biomarker, particularly for inflammation and neurodegeneration, where its decrease is expected. (25) For progranulin analysis, the E-EL-H1578 kit was used - range 62.50-4000 pg/ml - with dilution from 1 to 100.

Type III collagen is characteristic of muscle tissue and is synthesized from type III procollagen. Type III procollagen N-terminal peptide (P3NP) is released into the circulation during the late phases of collagen III synthesis and increases when skeletal muscle remodeling is activated. In this case, increased levels of P3NP were observed during normal increases in lean body mass in response to resistance exercise in adult males and also in the natural process during puberty. Type III procollagen was analyzed as a biomarker of anabolism. (27,64,65) For type III procollagen evaluation, the E-EL-H0182 kit was used - range 0.16-10 ng/mL - with a dilution of 1 to 20.

GDF-15 is a cytokine that functions as a biomarker of inflammation and mitochondrial function. It has the potential to be a marker of aging, muscle loss, reduced functional capacity and cardiovascular disease. (25) For GDF-15 evaluation, the E-EL-H0080 kit was used - range 23.44-1500 pg/mL - with a dilution of 1 to 10.

Compound 3MH is a product of the breakdown of contractile proteins and is a marker of muscle degeneration. The 3MH was evaluated in two ways: by ELISA test in the 30 participants. The kit used was EH4250- 3MH – range - 6.25-400nmol/ml - with dilution (1/2), another way was the evaluation by labeled isotope performed in 10 participants as described below: In the first 24 h after the stroke, and on the 7th day of hospitalization, the patient had blood samples collected for residual 3-methylhistidine evaluation. The participant received 50 mL of a solution containing 10 mg of 99.1% pure D3-methylhistidine (Cambridge Isotope Laboratories, Andover, MA, USA). After 18 h, three 10-mL blood samples were collected at 1-h intervals. Of 30 patients, 10 will be included in the analysis. The samples were sent to the Centre for Musculoskeletal Aging Research & NIHR Nottingham BRC (Clinical, Metabolic, and Molecular Physiology research group, University of Nottingham, Royal Derby Hospital Centre, Derby, UK). The ratio between labeled and unlabeled 3-methylhistidine was evaluated using liquid chromatography-mass spectrometry. (66–68)

The homeostasis assessment model of insulin resistance (HOMA-IR) was calculated from the values of fasting glucose and insulin. This index can provide an indirect measure of insulin resistance and has been used frequently in clinical studies because of its easy

application, speed, and low cost, showing good correlation with tests considered the gold standard for this purpose, such as the hyperinsulinemic clamp. (69)

4.9.8 ASSESSMENT OF ANXIETY AND DEPRESSION

The Hospital Anxiety and Depression Scale was used to screen for anxiety and depression and assess the severity of mood disorders 90 days after stroke. The scale contains 14 multiple-choice questions. It consists of two subscales, for anxiety and depression, with seven items each. The overall score on each subscale ranges from 0 to 21. Scores between 0 and 7 mean the absence of depressive or anxious symptoms; scores between 8 and 10 mean a possible case of depression or anxiety; and from 11 to 21 as a probable case of anxiety or depression. (70)

4.9.9 QUALITY OF LIFE ASSESSMENT

The EuroQol-5D was used to assess the individuals' perceptions of quality of life through five domains of mobility, personal care, usual activities, pain/discomfort, and anxiety/depression; the higher the value, the worse the quality of life perception. At the end of the questionnaire, the patient had to indicate their health status using an ordinal scale from 0 to 100; the closer to 0 the worse their health status, and the closer to 100 the better their health status. (71)

4.9.10 ASSESSMENT OF EATING HABITS

The food record of the 7 days of hospitalization of the patients was carried out, in which the percentage of consumption in each meal was recorded daily. Retrospective dietary instruments were applied: Food Frequency Questionnaire (FFQ) and 24-hour recall 90 days after the stroke.

The FFQ consists of a checklist of a specific number of foods that were developed by Wiehl, in 1960, and is frequently used in studies, mainly epidemiological, because it is a low-cost and easy-to-apply instrument, routinely used to associate diet with the occurrence of chronic diseases. (72)

The 24-hour recall consists of quantifying all food consumption in the 24 hours before the visit and can describe a wide variety of foods, for this reason, it is widely used to compare the average intake of calories and nutrients of different groups of people or different moments of the same group. As well as FFQ, the 24-hour recall is an easy-to-apply, low-cost instrument. (72)

4.10 STATISTICAL ANALYSIS

The Full Analysis Set (FAS) comprised all randomized subjects who received at least one dose of creatine. The subjects contributed to the analysis as randomized. The FAS was used for efficacy analysis instead of intention-to-treat analysis.

The Per-Protocol (PP) population comprised all subjects in the FAS who didn't have any major protocol deviations (including but not limited to violation of inclusion and exclusion criteria), and who had at least good compliance as reported in the eCRF and for whom the primary endpoint derived. The PP was used for the analysis of the primary endpoint.

The Safety Analysis Set included all subjects who received at least one dose of creatine or placebo. The subjects contributed to the analysis as treated. The Safety Analysis Set was used for the evaluation of safety endpoints and the primary endpoint. Missing values were treated mathematically by multiple imputations.

All data were analyzed using Stata SE version 15.0 (StataCorp LLC., College Station, TX, USA).

Planned statistical methods

- Statistical considerations baseline was defined as the randomization visit and the end of the trial was defined as the final visit. Categorical data was summarized by treatment group, using the number and percentages of subjects.
- For the calculation of percentages, the denominator was the number of subjects in the analysis set.
- Continuous data were presented using the number of subjects, mean, standard deviation, median, lower quartile, upper quartile, minimum, and maximum.

Both the absolute values and the change from baseline were presented. The treatment group and visit were presented descriptive statistics for all endpoints (when applicable).

All statistical tests were carried out as two-sided and performed on a 5% significance level. Estimated treatment differences and 95% confidence intervals were presented together with the corresponding p-value.

All safety endpoints were presented using descriptive statistics and no formal statistical tests was applied.

- The evaluation of continuous variables of the characteristics between the groups was analyzed by the t-test or Mann-Whitney.
- The evaluation of categorical variables of the characteristics between the groups was analyzed using Chi-square or Fisher Exact.
- The assessment of variation of serum levels of biomarkers, muscle mass, and strength between groups and time were done by the mixed regression models. The data were modelled according to the GMM (Generalized Mixed Models) using normal and gamma distributions. The chosen model had the lowest AIC (Akaike Information Criterion) value.
- Kaplan-Meier survival curve was used to show the survival in the groups within 90 days. Considering day zero as the day of randomization and entering into the study, patients related the final date they had each outcome. This is appropriate as it takes into account what happened during the course of the study. P-values were obtained through proportional Cox regression models and a value of 5% was considered statistically significant.
- All adverse events were summarized. The summaries included the number of events, the number of subjects, and the proportion of subjects reporting these events and were tabulated by system organ class.

Sensitivity analysis

The primary analysis was repeated using the PP and safety analysis set. We performed analysis using only participants with specific symptoms to each symptoms.

4.11 DATA MONITORING

The study follows the schedule of the Research Ethics Committee of Sao Paulo State University with the submission of partial reports to assess the progress and conduct of the study. At the end of the study, the final report will be sent with all the results. As this is an intervention with a low risk of serious adverse events, with a supplement known to be safe at

the doses administered in the study, interim analyzes are not foreseen before the end of the study.

Non-serious adverse events such as diarrhea, dyspepsia, and abdominal pain related to supplementation were analyzed individually and discussed with the assisting medical team to assess what action will be taken. Serious adverse events related or not to the intervention, such as death, acute kidney injury, infection with prolonged hospitalization, and hospitalization for any cause was reported to the institution's Research Ethics Committee within 24 h of their occurrence. All adverse events were summarized. The summaries included the number of events, the number of subjects, and the proportion of subjects reporting these events.

Monthly team meetings were held with the principal investigator to align the protocol, verify the progress of the study, and ensure compliance with deadlines.

4.12 ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Research Ethics Committee from Botucatu Medical School according to the Consubstantiated Opinion no. 2.421.189 (CAAE: 79844317.3.0000.5411) on December 07, 2017. (ANNEX A) This is version 4 of the June 8, 2022, of the protocol. Three amendments were sent to the Research Ethics Committee: 1 amendment was not approved (opinion number 5.307.562 of March 23, 2022) and 2 amendments were approved (opinion number 3.246.038 of April 5, 2019, and 5.456.441 of June 8, 2022). (ANNEX B) (ANNEX C)

The Brazilian Clinical Trials Registry (ReBEC) under the number RBR-9q7gg4, Registered in 21 January, 2019, <https://ensaiosclinicos.gov.br/rg/RBR-9q7gg4>.

Consent was requested to review participants' medical records and collect blood samples for biochemical dosages. Written, informed consent to participate was obtained from all participants. All participants will have access to care at the Botucatu Clinics Hospital through Brazil's unified health system in case there is any event caused by the study intervention. (APPENDIX A)

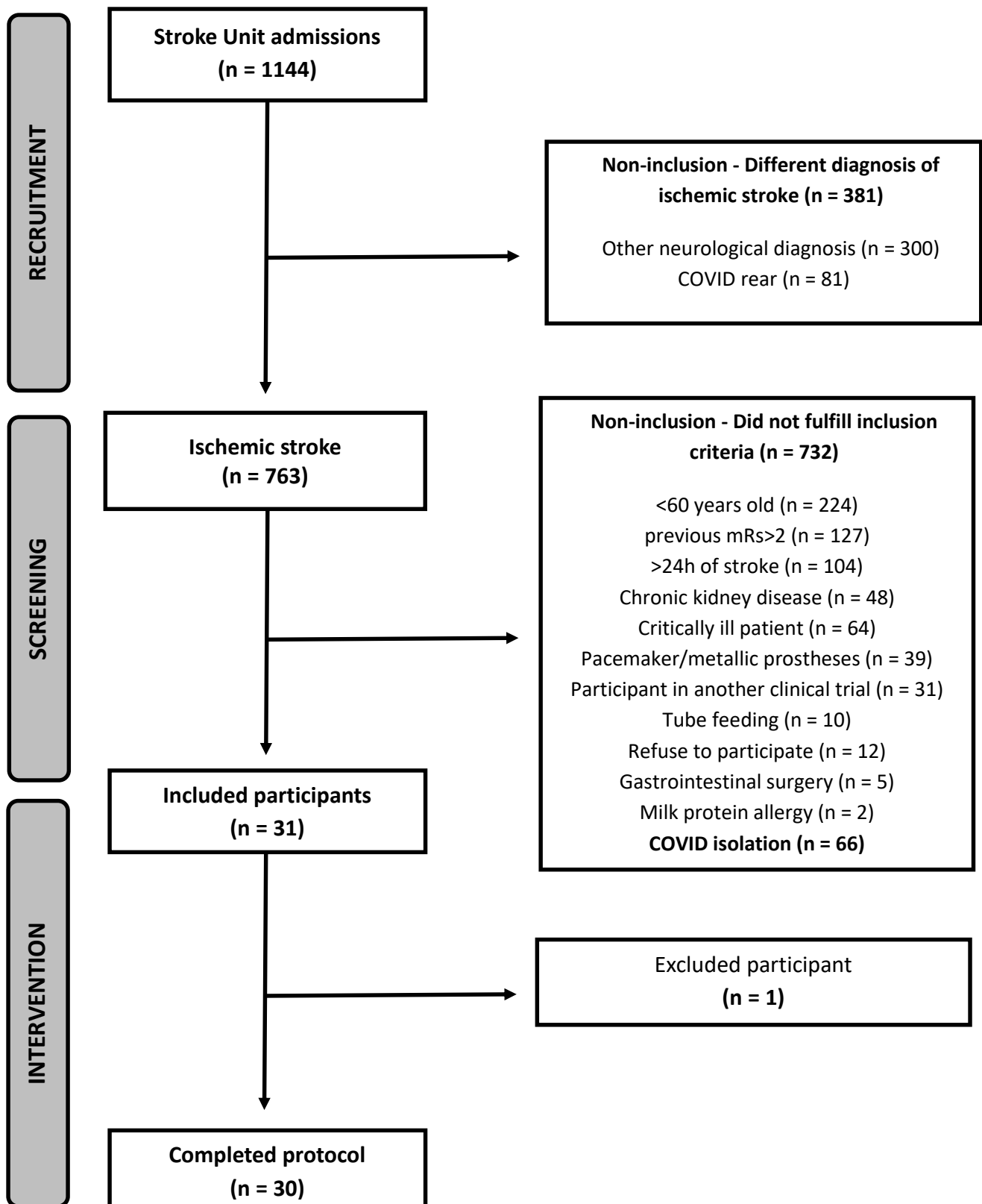
5 RESULTS

Recruitment and inclusion of participants in the study

Recruitment started in July 2019 and ended in November 2022, during this period 1144 patients were admitted to the Stroke Unit. Of these, 763 had a diagnosis of ischemic stroke and 31 were included in the study. Of these, one participant was excluded after inclusion as he contracted COVID and needed to be transferred to another unit. Thus, 30 participants followed the study protocol. The patient recruitment and inclusion flowchart can be observed in Figure 3.

From 2020 until the end of recruitment, the Stroke Unit received patients from other specialties due to the transformation of several hospital wards into Care Units for COVID patients. For this reason, there was an increase in the number of admissions to the stroke unit, in addition, 66 patients who had an ischemic stroke were not screened for the study to ensure the safety of the team during social isolation.

Figure 3 - Flowchart of inclusion of participants in the ICaRUS Stroke Trial from July/2019 to November/2022



Characteristics of study participants compared between treatment and control groups at the baseline visit

Below, the descriptive results of the general characteristics, neurological characteristics, anthropometric measurements, body composition, muscle strength, functional capacity, and biochemical dosages at baseline visits were exposed. It was observed that there was no difference between the treatment and control groups in any of the analyzed characteristics, showing that the groups were homogeneous at the time of inclusion in the study.

The comparison between the general characteristics of the participants in the treatment and control groups at the time of inclusion in the study is shown in Table 1.

Table 1 - Comparison between treatment and control groups of the general characteristics of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit

	Treatment (n=15)	Control (n=15)	p
Male sex, N (%)	10.0 (66.7)	10.0 (66.7)	0.699
Age (years)	69.0 (64.0-79.0)	66.0 (63.0-73.0)	0.884
NRS 2002 $\geq$ 3, N (%)	8.0 (53.3)	9.0 (60.0)	1.000
Diabetes mellitus, N (%)	4.0 (26.7)	6.0 (40.0)	0.699
Arterial hypertension, N (%)	10.0 (66.7)	12.0 (80.0)	0.682
Dyslipidemia, N (%)	5.0 (33.3)	6.0 (40.0)	1.000
Previous MI, N (%)	4.0 (26.7)	2.0 (13.3)	0.651
Previous stroke, N (%)	1.0 (6.7)	2.0 (13.3)	1.000
Thrombolysis, N (%)	6.0 (40.0)	6.0 (40.0)	0.709
Insulin use, N (%)	5.0 (33.3)	3.0 (20.0)	0.682
Statin use, N (%)	5.0 (33.3)	2.0 (13.3)	0.390
Dysphagia, N (%)	3.0 (20.0)	6.0 (40.0)	0.427
FOIS, N (%)	6.0 (40.0)	6.0 (40.0)	0.146

NRS: Nutritional risk screening; **MI:** Acute myocardial infarction; **mRs:** Modified Rankin Scale; **FOIS:** Functional Oral Intake Scale. Performed Chi-square or Fisher Exact test to evaluate categorical variables and the results were expressed as number and percentage. Performed Mann-Whitney for age and the result was expressed as median and 25th-75th percentiles.

The comparison between the neurological characteristics of the participants in the treatment and control groups at the time of inclusion in the study is shown in Table 2.

Table 2 - Comparison between treatment and control groups of the neurological characteristics of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit

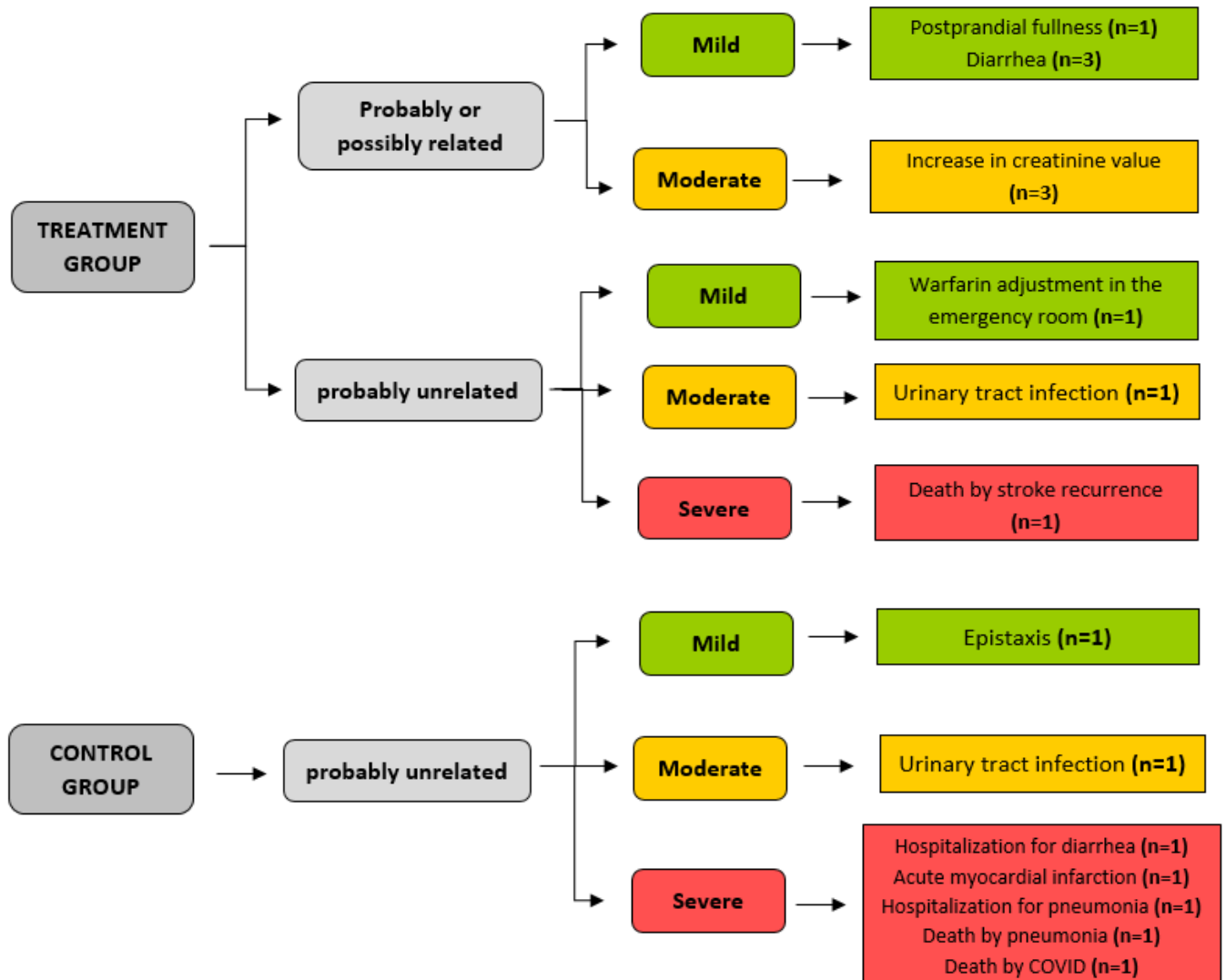
	Treatment (n=15)	Control (n=15)	P
ASPECTS score	10.0 (8.0-10.0)	8.0 (8.0-9.0)	0.126
NIHSS at admission	7.0 (4.0-13.0)	8.0 (6.0-12.0)	0.546
<i>Bamford classification</i>			
LACS, N (%)	7.0 (23.3)	6.0 (20.0)	0.847
PACS, N (%)	4.0 (13.3)	6.0 (20.0)	0.847
TACS, N (%)	2.0 (6.7)	1.0 (3.3)	0.847
POCS, N (%)	2.0 (6.7)	2.0 (6.7)	0.847
<i>Toast classification</i>			
Cardioembolism, N (%)	3.0 (10.0)	4.0 (13.3)	0.411
Large-vessel, N (%) atherothrombosis, N (%)	4.0 (13.3)	2.0 (6.7)	0.411
Undetermined causes, N (%)	8.0 (26.7)	7.0 (23.3)	0.411
Small-vessel disease, N (%)	0.0 (0.0)	2.0 (6.7)	0.411

ASPECTS: Alberta Stroke Program Early CT Score; **NIHSS:** National Institutes of Health Stroke Scale; **LACS:** Lacunar syndrome; **PACS:** Partial anterior circulation syndrome; **TACS:** Total anterior circulation syndrome; **POCS:** Posterior circulation syndrome. Performed Mann-Whitney for ASPECTS score and NIHSS and the results were expressed as median and 25th-75th percentiles. Performed Chi-square or Fisher Exact test to evaluate categorical variables and the results were expressed as number and percentage.

Concerning the adverse events that occurred during the study, 3 participants who received creatine had symptoms of diarrhea, and 1 participant complained of postprandial fullness after consuming the supplement, with rapid improvement after adjusting the time of administration. Three participants in the treatment group had a significant increase in creatinine values (above 60% of the initial value) at the end of the 7 days of intervention, with

rapid resolution after hydration. All adverse events that occurred during participation in the ICaRUS Stroke Trial are shown in Figure 4.

Figure 4 - Adverse events that occurred during participation of older people in the ICaRUS Stroke Trial



Source: material prepared by the author.

Regarding early mobility training, the number of sessions during the intervention period was similar in both groups, being 6.1 ± 2.9 in the treatment group and 5.0 ± 3.0 in the control group ($p=0.308$).

The comparison between the body composition of the participants in the treatment and control groups at the time of inclusion in the study is shown in Table 3.

Table 3 - Comparison between treatment and control groups of the body composition of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit

	Treatment (n=15)	Control (n=15)	p
Body weight (kg)	69.8±14.1	68.6±12.0	0.816
Body Mass Index (kg/m ²)	25.7±4.1	26.0±4.8	0.850
BBM thickness (US) - affected side (mm)	25.6±6.8	25.2±4.8	0.862
BBM thickness (US) - unaffected side (mm)	22.5±5.2	25.4±6.3	0.205
RFM thickness (US) - affected side (mm)	15.7 (12.2-21.4)	14.3 (11.4-22.0)	0.982
RFM thickness (US) - unaffected side (mm)	15.9±4.2	16.9±5.8	0.648
SMM affected arm (kg)	1.44 (0.9-1.8)	1.4 (1.2-1.5)	0.908
SMM unaffected arm (kg)	1.5 (0.9-2.1)	1.4 (1.3-1.7)	0.747
SMM affected leg (kg)	4.1 (3.3-4.9)	4.0 (3.6-4.9)	0.854
SMM unaffected leg (kg)	3.9 (3.1-5.2)	3.8 (3.6-4.6)	0.963
Fat Mass Index (kg/m ²)	7.9±6.6	7.3±3.5	0.717
Fat Free Mass Index (kg/m ²)	18.5 (16.4-21.4)	18.5 (17.7-19.9)	0.565
ECW/TBW (%)	44.5 (42.5-47.6)	43.6 (42.2-45.8)	0.475
Phase angle	5.5 (4.6-6.7)	5.6 (5.3-6.8)	0.488
AMMI (kg/m ²)	11.0±5.3	10.9±2.6	0.962
Visceral fat (l)	3.5 (2.7-4.3)	2.9 (2.4-4.7)	0.661

BBM: Biceps brachii muscle; **US:** Ultrasound; **RFM:** Rectus femoris muscle; **SMM:** Skeletal muscle mass; **ECW:** Extracellular water; **TBW:** Total body water; **AMMI:** Appendicular muscle mass index. Performed t-test or Mann-Whitney to evaluate continuous variables and the results were expressed as mean±standard or median and 25th-75th percentiles.

The comparison between the muscle strength of the participants in the treatment and control groups at the time of inclusion in the study is shown in Table 4.

Table 4 - Comparison between treatment and control groups of the muscle strength of older people hospitalized for ischemic stroke who participated in the ICarUS Stroke Trial at baseline visit

	Treatment (n=15)	Control (n=15)	P
Handgrip affected side (kgf)	0.0 (0.0-22.0)	0.0 (0.0-19.0)	0.982
Handgrip unaffected side (kgf)	22.8±12.5	17.9±12.7	0.300
NIHSS affected leg	1.0 (0.0-3.0)	0.0 (0.0-3.0)	0.659
NIHSS unaffected leg	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.577

NIHSS: National Institutes of Health Stroke Scale. Performed t-test or Mann-Whitney and the results were expressed as mean±standard or median and 25th-75th percentiles.

To compare the functional capacity between the treatment and control groups at the baseline visit, the Modified Rankin scale was applied and it was observed that the median score in the treatment group (n=15) was 4.0 (1.0-5.0) and in the control group (n=15) was 5.0 (1.0-5.0) (p=0.335).

The comparison between treatment and control groups of biochemical exams of patients hospitalized for ischemic stroke at the time of inclusion in the study is shown in Table 5.

Table 5 - Comparison between treatment and control groups of the biochemical exams and bio markers of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at baseline visit

	Treatment (n=15)	Control (n=15)	P
Hematocrit (g/dl)	41.8 (38.7-42.9)	41.7 (41.0-43.9)	0.419
Hemoglobin (%)	13.8 (13.0-14.2)	13.9 (13.5-14.1)	0.349
Lymphocytes (%)	20.2±8.8	21.9±5.9	0.528
Total cholesterol (mg/dl)	181.2±38.2	188.1±44.3	0.653
HDL cholesterol (mg/dl)	43.0 (33.0-65.0)	43.0 (31.0-58.0)	0.967
LDL cholesterol (mg/dl)	111.1±35.1	118.1±37.7	0.599
Triglycerides (mg/dl)	118.0 (68.0-159.0)	88.0 (83.0-135.0)	0.604
Total proteins (g/dl)	6.4±0.4	6.9±0.7	0.106
Albumin (g/dl)	3.6±0.4	3.8±0.4	0.408
C-reactive protein (mg/dl)	0.6 (0.5-2.2)	0.5 (0.5-1.9)	0.963
Urea (mg/dl)	45.9±16.8	33.2±10.2	0.019
Creatinofosfoquinase (mg/dl)	84.0 (52.0-133.0)	98.0 (67.0-143.0)	0.561
HbA1C (%)	5.9 (5.5-6.2)	5.8 (5.8-6.4)	0.484
Creatinine (mg/dl)	0.9 (0.9-1.0)	0.9 (0.8-1.1)	0.884
Glucose (mg/dl)	102.0 (90.0-135.0)	123.0 (106.0-141.0)	0.135
Interleukin-6 (pg/ml)	2.5 (2.0-3.4)	2.5 (2.2-4.0)	0.480
GDF-15 (pg/ml)	637.5±256.6	578.1±202.7	0.488
Insulin (μUI/mL)	9.0 (7.7-19.4)	14.9 (3.2-22.2)	0.709
IGF-1 (ng/ml)	11.6 (6.2-45.2)	10.6 (6.5-41.7)	0.917
Type III procollagen (ng/ml)	21.98 (14.1-33.3)	25.7 (17.4-41.0)	0.534
Progranulin (pg/ml)	7001.9 (6263.5-8589.1)	7465.4 (5576.8-9583.7)	0.967
HOMA-IR	2.5 (1.6-6.4)	5.4 (1.5-6.9)	0.561

HDL: High-density lipoprotein; **LDL:** Low-density lipoprotein; **HbA1C:** Hemoglobin A1C; **GDF-15:** Growth/Differentiation Factor-15; **IGF-1:** Insulin-like growth factor 1; **HOMA-IR:** Homeostasis assessment model of insulin resistance. Performed t-test or Mann-Whitney and the results were expressed as mean±standard or median and 25th-75th percentiles.

Evaluation of the influence of creatine treatment on body composition, muscle strength, functional capacity, and biochemical tests

Creatine supplementation during 7 days of hospitalization in older people who experienced an ischemic stroke did not impact functional capacity and strength, but increased appendicular muscle mass in men compared to the control group.

The results of the influence of creatine supplementation during seven days of hospitalization on the muscle mass of older people in the acute phase of stroke can be seen in Table 6. The body composition variables of older people who participated in the ICarUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo are shown in Figure 5.

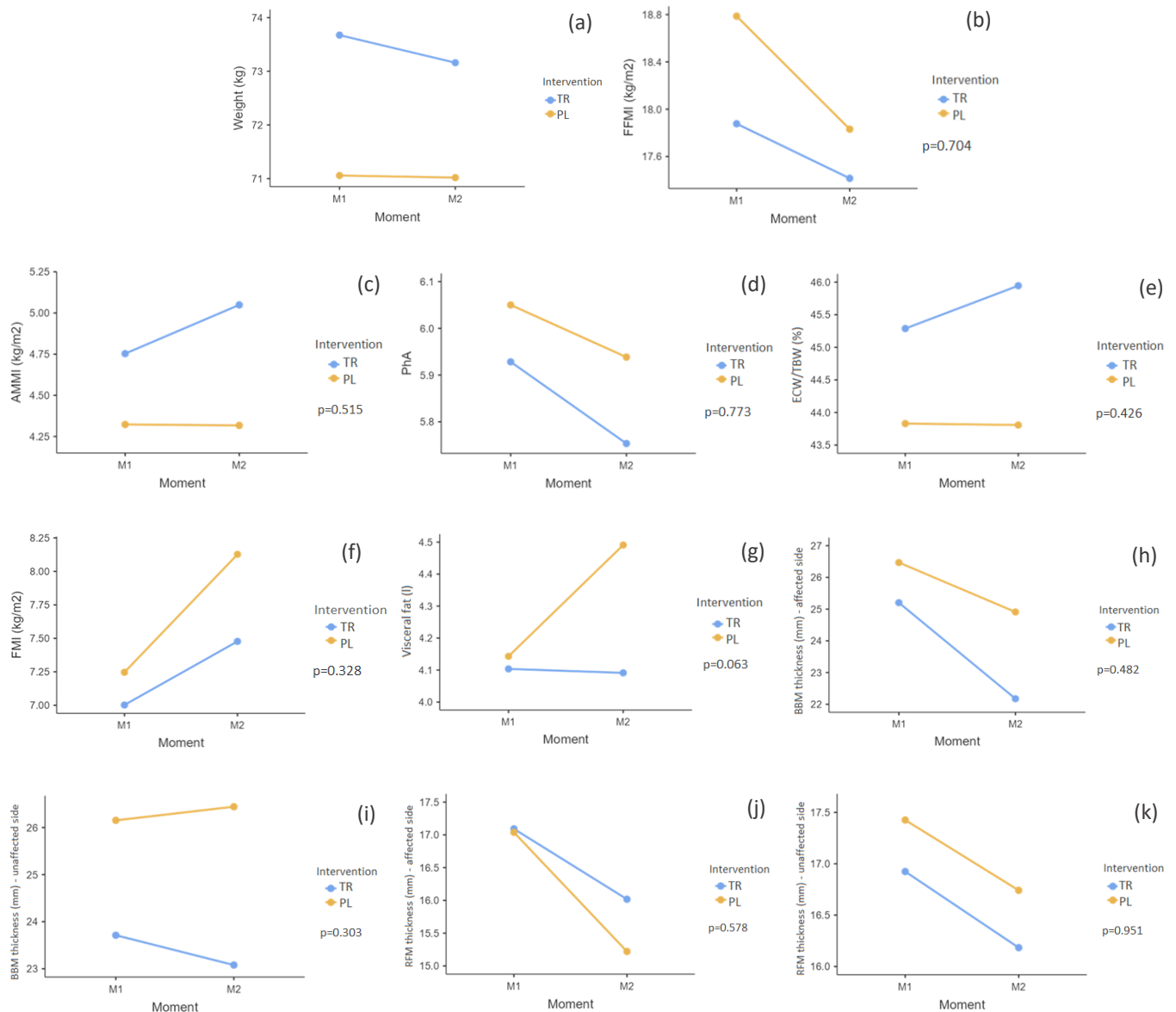
By adding sex in the models of body composition variables, there were no changes in the patterns. Except for AMMI, where an increase in values was observed in men and not in women. The results of this finding can be found in Table 7 and the graphs of this interaction in Figure 6.

Table 6 - Evaluation of the influence of creatine treatment on muscle mass in older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial (n=30)

Outcomes	Variables	Estimate	95% Confidence interval		p
			Lower	Uper	
Weight	Interaction of time x treatment	0.538	-0.686	1.761	0.389
	Treatment	-2.228	-14.425	9.969	0.720
FFMI	Interaction of time x treatment	-0.494	-3.01	2.026	0.704
	Treatment	0.663	-1.59	2.919	0.570
AMMI	Interaction of time x treatment	-0.302	-2.524	1.92	0.790
	Treatment	-0.581	-2.330	1.17	0.515
Phase angle	Interaction of time x treatment	0.0632	-0.367	0.4931	0.773
	Treatment	0.1533	-0.721	1.0280	0.731
ECW/TBW	Interaction of time x treatment	-0.680	-2.354	0.994	0.426
	Treatment	-1.797	-5.321	1.728	0.318
Fat mass index	Interaction of time x treatment	0.406	-0.390	1.20	0.328
	Treatment	0.447	-2.720	3.61	0.784
Visceral fat	Interaction of time x treatment	0.360	-0.0193	0.740	0.063
	Treatment	0.220	-1.2898	1.729	0.776
BBM thickness - affected side	Interaction of time x treatment	1.47	-2.62	5.558	0.482
	Treatment	2.00	-3.28	7.279	0.458
BBM thickness - unaffected side	Interaction of time x treatment	0.923	-0.834	2.680	0.303
	Treatment	2.901	-2.323	8.125	0.276
RFM thickness - affected side	Interaction of time x treatment	-0.744	-3.36	1.876	0.578
	Treatment	-0.425	-4.80	3.952	0.849
RFM thickness - unaffected side	Interaction of time x treatment	0.0566	-1.74	1.855	0.951
	Treatment	0.5298	-3.81	4.866	0.811

FFMI: Fat free mass index; **AMMI:** Appendicular muscle mass index; **ECW:** Extracellular water; **TBW:** Total body water; **BBM:** Biceps brachii muscle; **RFM:** Rectus femoris muscle. Performed Generalized Mixed Models using normal and gamma distributions.

Figure 5 - Body composition variables of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo



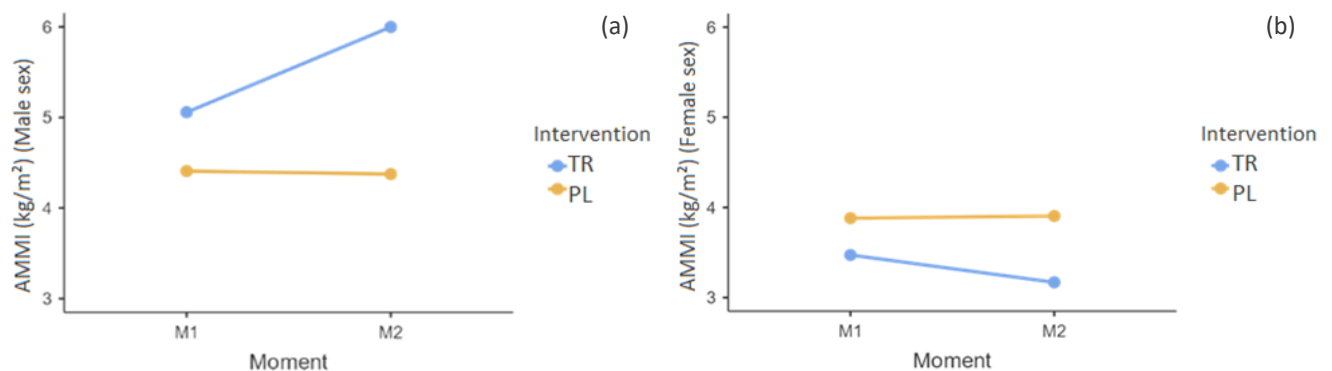
Source: material prepared by the author. **TR**: Treatment; **PL**: Placebo; **M1**: Moment 1 (24h after stroke); **M2**: Moment 2 (7th day of hospitalization); **(b)**: FFMI: Fat free mass index; **(c)**: AMMI: appendicular muscle mass index; **(d)**: Phase angle; **(e)**: ECW: Extracellular water; TBW: Total body water; **(f)**: Fat mass index; **(h, i)**: BBM: Biceps brachii muscle; **(j, k)**: RFM: Rectus femoris muscle.

Table 7 - Evaluation of the influence of creatine treatment on appendicular muscle mass index of older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial corrected by sex (n=30)

Outcomes	Variables	Estimate	95% Confidence interval		p
			Lower	Uper	
AMMI corrected	Interaction of time x treatment	-0.324	-0.328	-0.321	< .001
by sex	Treatment	-0.282	-0.285	-0.279	< .001

AMMI: appendicular muscle mass index. Performed Generalized Mixed Models using normal and gamma distributions.

Figure 6 - Appendicular muscle mass index of older during participation in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo corrected by sex



Source: material prepared by the author. **TR:** Treatment; **PL:** Placebo; **M1:** Moment 1 (24h after stroke); **M2:** Moment 2 (7th day of hospitalization). **(a,b):** AMMI: appendicular muscle mass index.

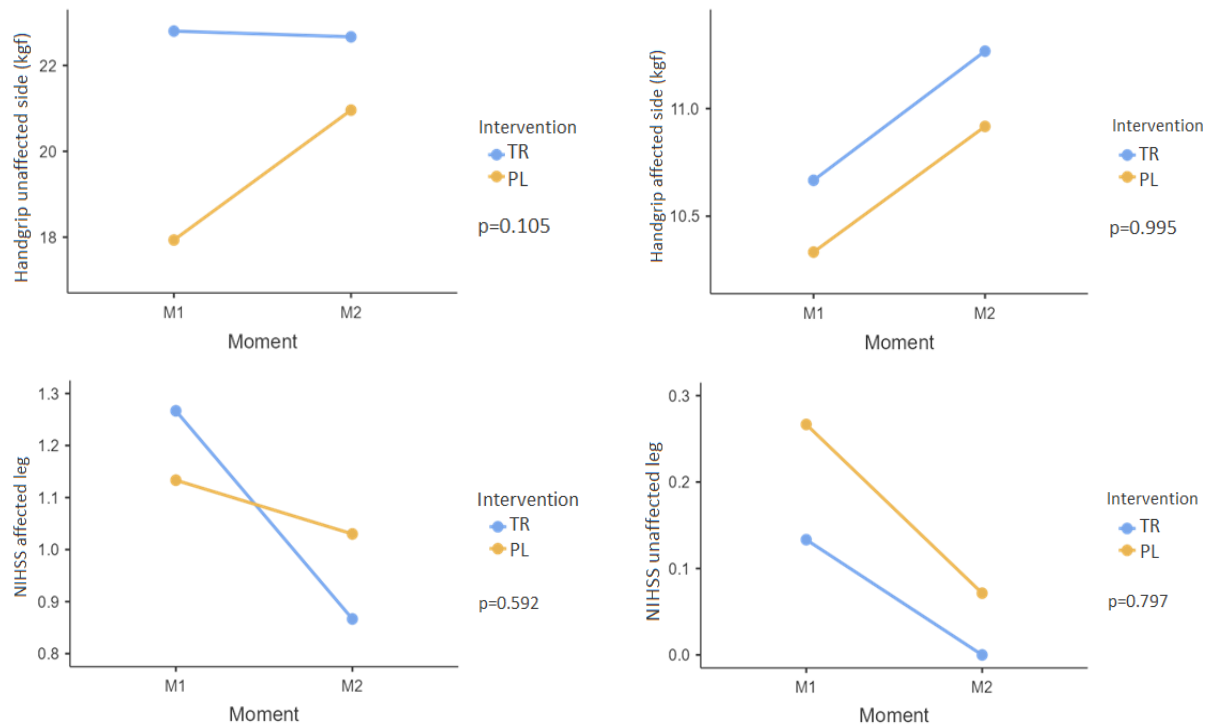
The results of the influence of creatine supplementation during seven days of hospitalization on the muscle strength of older people in the acute phase of stroke can be seen in Table 8. The muscle strength variables of older people who participated in the ICaRUS Stroke Trial is shown in Figure 7.

Table 8 - Evaluation of the influence of creatine treatment on muscle strength in older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial (n=30)

Outcomes	Variables	Estimate	95% Confidence interval		p
			Lower	Uper	
Handgrip unaffected side	Interaction of time x treatment	3.16	-0.535	6.85	0.105
	Treatment	-3.29	-12.411	5.84	0.486
Handgrip affected side	Interaction of time x treatment	-0.0163	-5.00	4.97	0.995
	Treatment	-0.3415	-10.36	9.67	0.947
NIHSS affected leg	Interaction of time x treatment	0.2967	-0.776	1.370	0.592
	Treatment	0.0150	-0.920	0.950	0.975
NIHSS unaffected leg	Interaction of time x treatment	-0.0619	-0.531	0.4069	0.797
	Treatment	0.1024	-0.132	0.3368	0.396

NIHSS: National Institute of Health Stroke Scale. Performed Generalized Mixed Models using normal and gamma distributions.

Figure 7 - Muscle strength variables of older people during participation in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo



Source: material prepared by the author. **TR:** Treatment; **PL:** Placebo; **M1:** Moment 1 (24h after stroke); **M2:** Moment 2 (7th day of hospitalization); **NIHSS:** National Institutes of Health Stroke Scale.

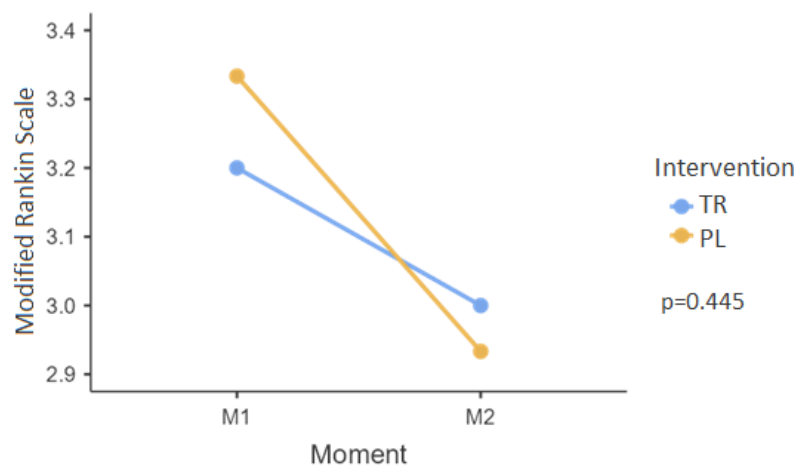
The results of the effectiveness of creatine supplementation during seven days of hospitalization on the functional capacity of older people in the acute phase of stroke can be seen in Table 9. Functional capacity by Modified Rankin Scale of older people who participated in the ICaRUS Stroke Trial is shown in Figure 8.

Table 9 - Evaluation of the influence of creatine treatment on functional capacity in older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial (n=30)

Outcomes	Variables	Estimate	95% Confidence interval		p
			Lower	Uper	
Modified Rankin Scale	Interaction of time x treatment	-0.2000	0.706	0.3061	0.445
	Treatment	0.0333	1.263	1.3294	0.960

Performed Generalized Mixed Models using normal and gamma distributions.

Figure 8 - Functional capacity by Modified Rankin Scale of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo



Source: material prepared by the author. **TR:** Treatment; **PL:** Placebo; **M1:** Moment 1 (24h after stroke); **M2:** Moment 2 (7th day of hospitalization).

The results on the effectiveness of creatine supplementation during seven days of hospitalization on biochemical tests of older people in the acute phase of stroke can be seen in Table 10. The biochemical markers results of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th of intervention with creatine versus placebo is shown in Figure 9.

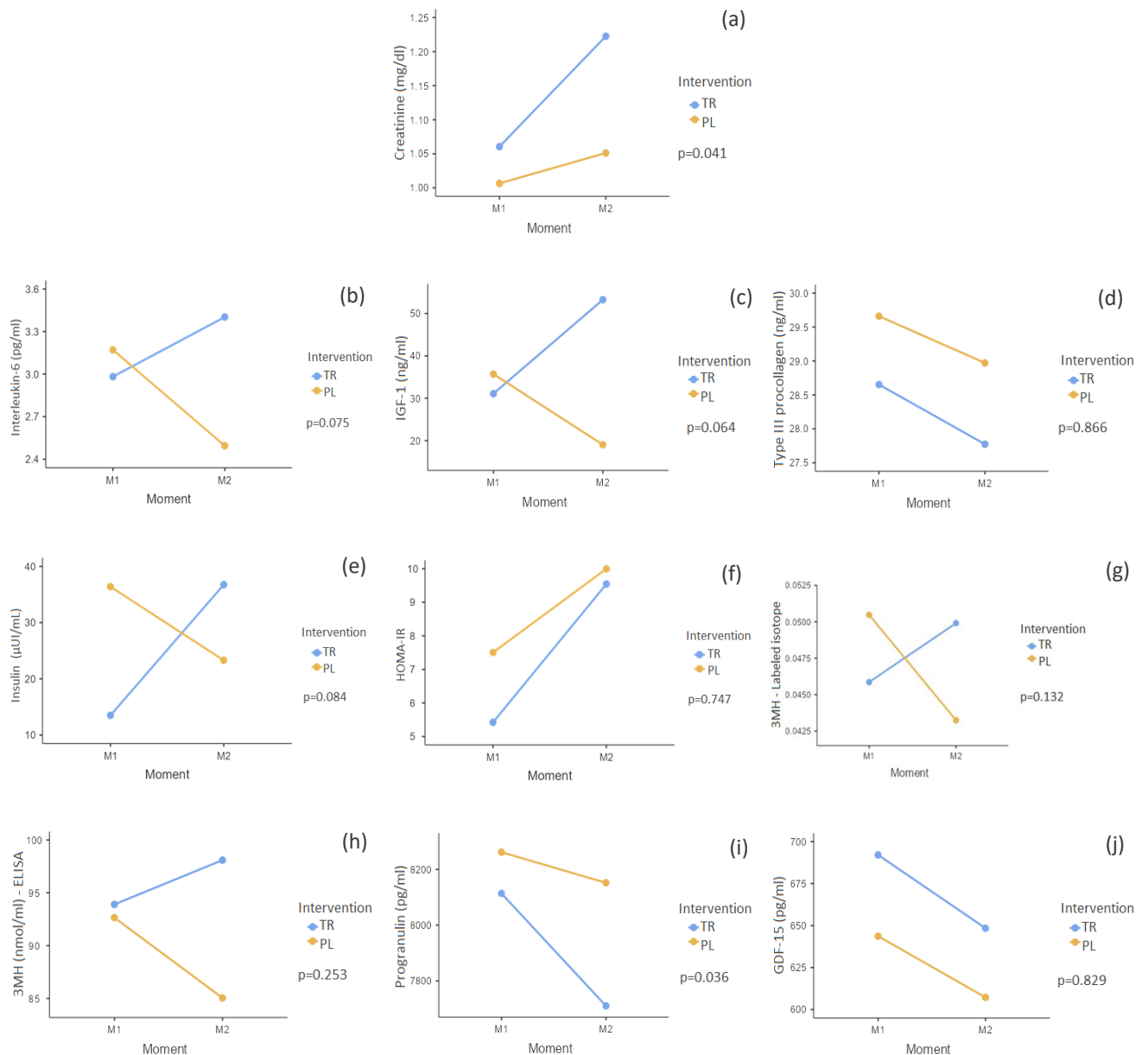
By adding sex in the models of IGF-1, IL-6, and 3-MH in a sample isolated by ELISA, there were no changes in the patterns. On the 3MH biomarker by labeled isotope, it is observed that there is an interaction between treatment and moment, but not with sex. The results of this finding can be found in Table 11, and the graphs of this interaction in Figure 10.

Table 10 - Evaluation of the influence of creatine treatment on biochemical markers of older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial (n=30)

Outcomes	Variables	Estimate	95% Confidence interval		p
			Lower	Uper	
Creatinine	Interaction of time x treatment	-0.118	-0.2302	-0.00493	0.041
	Treatment	-0.113	-0.3561	0.13067	0.364
Interleukin-6	Interaction of time x treatment	-0.3716	-0.780	0.0368	0.075
	Treatment	-0.1245	-0.641	0.3919	0.637
IGF-1	Interaction of time x treatment	-38.8	-79.9	2.32	0.064
	Treatment	-14.79	-35.6	6.06	0.164
Type III procollagen	Interaction of time x treatment	0.190	-2.02	2.401	0.866
	Treatment	1.103	-10.12	12.326	0.847
Insulin	Interaction of time x treatment	-36.34	-77.6	4.92	0.084
	Treatment	4.71	-15.9	25.36	0.655
HOMA-IR	Interaction of time x treatment	-1.63	-11.52	8.26	0.747
	Treatment	1.27	-4.37	6.91	0.660
3MH - labeled isotope	Interaction of time x treatment	-0.01128	-0.02595	0.00339	0.132
	Treatment	-0.00103	-0.03158	0.02953	0.948
3MH - ELISA	Interaction of time x treatment	-11.79	-32.0	8.44	0.253
	Treatment	-7.15	-35.8	21.54	0.625
Progranulin	Interaction of time x treatment	294	19.7	567.4	0.036
	Treatment	295	-75.3	664.6	0.118
GDF-15	Interaction of time x treatment	7.19	-58.0	72.38	0.829
	Treatment	-44.79	-212.2	122.58	0.600

GDF-15: Growth/Differentiation Factor-15; **IGF-1:** Insulin-like growth factor 1; **3MH:** 3-Methylhistidine; **ELISA:** Enzyme-linked immunoassay; **HOMA-IR:** Homeostasis assessment model of insulin resistance. Performed Generalized Mixed Models using normal and gamma distributions.

Figure 9 - Biochemical markers of older people who participated in the ICaRUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo



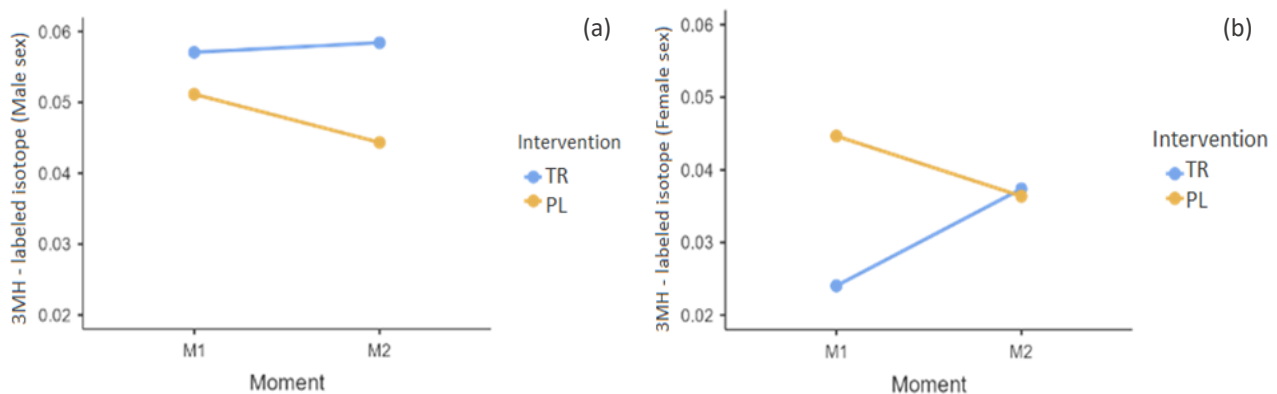
Source: material prepared by the author. **TR**: Treatment; **PL**: Placebo; **M1**: Moment 1 (24h after stroke); **M2**: Moment 2 (7th day of hospitalization); **(c)**: IGF-1: Insulin-like growth factor 1; **(f)**: HOMA-IR: Homeostasis assessment model of insulin resistance; **(g)**: 3MH: 3-Methylhistidine by labeled isotope; **(h)**: 3MH: 3-Methylhistidine by ELISA; ELISA: Enzyme-Linked Immunosorbent Assay; **(j)**: GDF-15: Growth/Differentiation Factor-15.

Table 11 - Evaluation of the influence of creatine treatment on the 3MH biomarker by labeled isotope of older people hospitalized for stroke after seven days of supplementation during participation in the Icarus Stroke Trial corrected by sex (n=10)

Outcomes	Variables	Estimate	95% Confidence interval		p
			Lower	Uper	
3MH - labeled isotope corrected by sex	Interaction of time x treatment	-0.01490	-0.02963	-1.74e-4	0.047
	Treatment	-9.98e-5	-0.00746	0.00726	0.979

3MH: 3-Methylhistidine. Performed Generalized Mixed Models using normal and gamma distributions.

Figure 10 - 3MH biomarker by labeled isotope of older people who participated in the ICarUS Stroke Trial in the first 24 hours after stroke and on the 7th day of intervention with creatine versus placebo corrected by sex

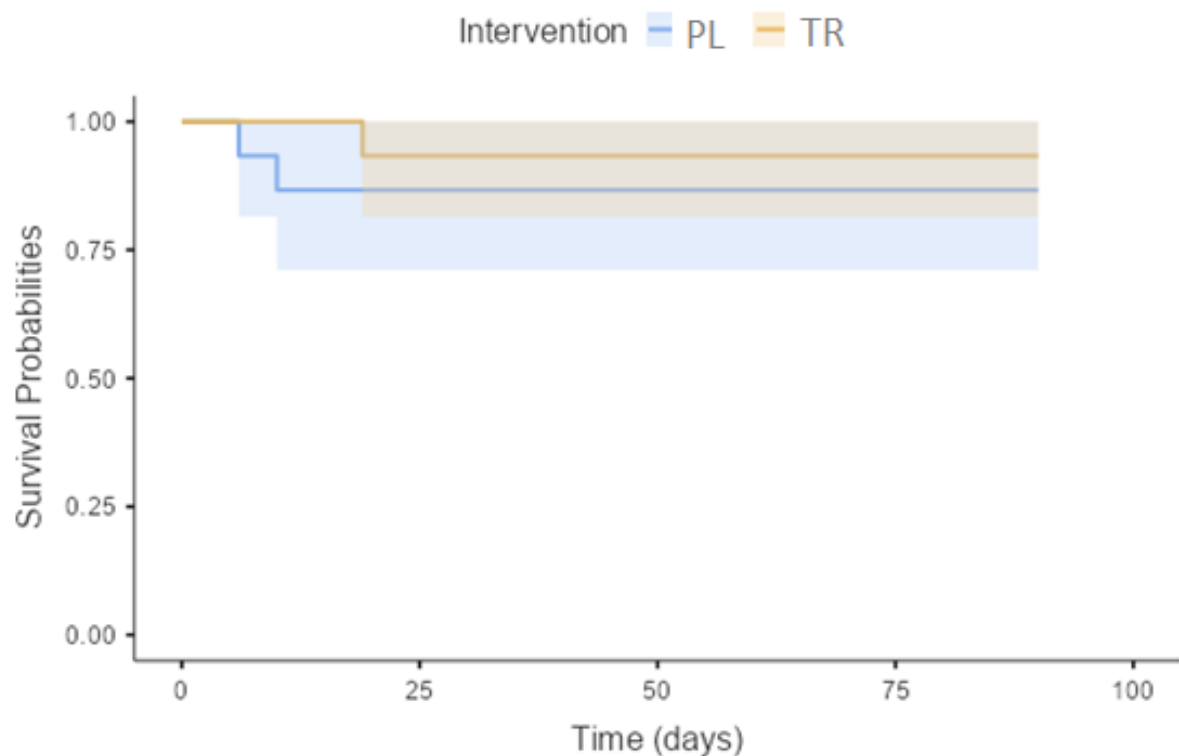


Source: material prepared by the author. **TR:** Treatment; **PL:** Placebo; **M1:** Moment 1 (24h after stroke); **M2:** Moment 2 (7th day of hospitalization); (a, b): 3MH: 3-Methylhistidine.

Assessment of mortality during study participation

The Kaplan-Meier survival curve was constructed to show the 90-day survival of the groups and it was verified that there was no difference between the participants in the treatment and control groups in terms of mortality, as can be seen in Figure 11. Three participants died during the study period. There was no difference in mortality between treatment and control groups ($p=0.523$) according to Cox regression, regardless of age, sex, and stroke severity over the 90 days.

Figure 11 Kaplan-Meier survival curve of the older people during participation in the Icarus Stroke Trial



Source: material prepared by the author. **PL:** Placebo; **TR:** Treatment.

Characteristics of study participants compared between treatment and control groups at the follow-up visit

Below, the descriptive results of the quality of life perception, anxiety/depression symptoms, and eating habits at the follow-up visit were exposed. It was observed that there

was no difference between the treatment and control groups in any of the analyzed characteristics.

The influence of creatine supplementation on muscle strength and functional capacity was assessed by the 30-second chair stand test and Timed Up and Go test which were performed on the seventh day of hospitalization and at the follow-up visit 90 days after the stroke. Creatine did not influence these parameters and the graphs of this interaction are shown in APPENDIX B.

The results related to the perception of quality of life, anxiety, and depression that the participants reported in the follow-up visit 90 days after the stroke can be found in Table 12.

Table 12 - Comparison between treatment and control groups on the perception of quality of life, anxiety, and depression of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at the follow-up visit

	Treatment (n=15)	Control (n=15)	p
<i>EuroQol-5D</i>			
Total score	8.0 (7.0-10.0)	7.0 (6.0-10.5)	0.538
Self-rated health score	73.8±22.2	65.6±27.5	0.409
<i>HADS</i>			
Anxiety	3.0 (2.0-8.0)	3.0 (0.5-7.5)	0.679
Depression	1.0 (0.5-4.0)	6.0 (1.5-11.5)	0.063

EuroQol-5D: The European 5D Quality of life Scale; **HADS:** Hospital Anxiety and Depression Scale. Performed t-test or Mann-Whitney and the results were expressed as mean±standard or median and 25th-75th percentiles.

The results of the food intake of the study participants from the 7-day food record applied during the intervention period including supplementation provided during study participation to reach the target of 1.5g protein/kg body weight/day, can be found in Table 13.

Table 13 - Comparison between treatment and control groups of results from 7-day food record of older people with ischemic stroke during the participation in the ICaRUS Stroke Trial

	Treatment (n=15)	Control (n=15)	p
Energy (kcal/day)	1463.3±419.9	1660.3±292.9	0.147
Energy (kcal/ kg body weight /day)	21.5±7.1	24.8±6.1	0.178
Proteins (g/day)	104.7±21.1	102.9±18.0	0.816
Lipids (g/day)	35.1 (24.9-44.6)	42.4 (37.5-44.9)	0.178
Carbohydrates (g/day)	215.8±97.8	254.5±73.2	0.230

Performed t-test or Mann-Whitney and the results were expressed as mean±standard or median and 25th-75th percentiles.

The results regarding the participants eating habits from the 24-hour recall and FFQ applied at the follow-up visit 90 days after the stroke can be found in Tables 14 and 15.

Table 14 - Comparison between treatment and control groups of results from 24-hour Dietary Recall of older people hospitalized for ischemic stroke who participated in the ICaRUS Stroke Trial at the follow-up visit

	Treatment (n=13)	Control (n=13)	P
Energy (kcal/day)	1944.4±548.5	1544.1±581.4	0.084
Proteins (g/day)	88.2±27.9	77.9±37.0	0.435
Lipids (g/day)	55.4±24.7	47.1±17.8	0.331
Carbohydrates (g/day)	258.3±89.4	202.7±85.3	0.118

Performed t-test or Mann-Whitney and the results were expressed as mean±standard or median and 25th-75th percentiles.

Table 15 - Consumption of food groups by older people with ischemic stroke who participated in the ICaRUS Stroke Trial (n=26)

	Consumption	1-2 times a day	≥3 times a day	1-2 times a week	≥3 times a week	1-2 times a month
Milk and dairy products						
Whole milk, N (%)	16.0 (61.5)	12.0 (46.1)	2.0 (7.7)	2.0 (7.7)	0	0
Skim milk, N (%)	4.0 (15.4)	3.0 (11.5)	0	0	0	1.0 (3.8)
Yogurt, N (%)	11.0 (42.3)	2.0 (7.7)	0	5.0 (19.2)	1.0 (3.8)	3.0 (11.5)
Low-fat cheese, N (%)	16.0 (61.5)	2.0 (7.7)	1.0 (3.8)	5.0 (19.2)	2.0 (7.7)	6.0 (23.1)
Whole cheese, N (%)	7.0 (26.9)	1.0 (3.8)	0	3.0 (11.5)	0	3.0 (11.5)
Butter, N (%)	12.0 (46.2)	8.0 (30.8)	0	4.0 (15.4)	0	0
Breads, cereals and pasta						
Bread, N (%)	26.0 (100.0)	24.0 (92.3)	0	1.0 (3.8)	1.0 (3.8)	0
Cheese bread, N (%)	12.0 (46.2)	1.0 (3.8)	0	6.0 (23.1)	1.0 (3.8)	4.0 (15.4)
Crackers/Toast, N (%)	20.0 (76.9)	10.0 (38.5)	0	8.0 (30.8)	2.0 (7.7)	0
Rice, N (%)	26.0 (100.0)	25.0 (96.1)	0	0	1.0 (3.8)	0
Potato/Mashed potato, N (%)	21.0 (80.8)	3.0 (11.5)	0	13.0 (50.0)	4.0 (15.4)	1.0 (3.8)
Noodles, N (%)	22.0 (84.6)	1.0 (3.8)	0	18.0 (69.2)	1.0 (3.8)	2.0 (7.7)
Fruits and Juices						
Orange, N (%)	20.0 (76.9)	12.0 (46.2)	1.0 (3.8)	5.0 (19.2)	2.0 (7.7)	0
Banana, N (%)	26.0 (100.0)	14.0 (53.9)	9.0 (34.6)	0	3.0 (11.5)	0
Apple/Pear, N (%)	13.0 (50.0)	8.0 (30.8)	0	5.0 (19.2)	0	0
Papaya, N (%)	19.0 (73.1)	11.0 (42.3)	0	7.0 (26.9)	0	1.0 (3.8)
Mango, N (%)	16.0 (61.5)	7.0 (26.9)	0	8.0 (30.8)	0	1.0 (3.8)
Orange juice, N (%)	8.0 (30.8)	4.0 (15.4)	0	4.0 (15.4)	0	0
Fruit juice, N (%)	11.0 (42.3)	7.0 (26.9)	0	3.0 (11.5)	1.0 (3.8)	0
Beans						
Beans, N (%)	25.0 (96.1)	24.0 (92.3)	0	1.0 (3.8)	0	0
Vegetables						
Lettuce/Arugula, N (%)	21.0 (80.8)	12.0 (46.2)	0	7.0 (26.9)	1.0 (3.8)	1.0 (3.8)
Cabbage/Kale, N (%)	21.0 (80.8)	14.0 (53.9)	0	6.0 (23.1)	0	1.0 (3.8)
Carrot/Pumpkin, N (%)	23.0 (88.5)	14.0 (53.9)	0	7.0 (26.9)	0	2.0 (7.7)
Tomato, N (%)	23.0 (88.5)	14.0 (53.9)	0	7.0 (26.9)	0	2.0 (7.7)
Chayote/Zucchini, N (%)	20.0 (76.9)	13.0 (50.0)	0	5.0 (19.2)	0	2.0 (7.7)
Okra/Eggplant, N (%)	18.0 (69.2)	7.0 (26.9)	0	7.0 (26.9)	0	4.0 (15.4)
Homemade soup, N (%)	15.0 (57.7)	2.0 (7.7)	0	8.0 (30.8)	2.0 (7.7)	3.0 (11.5)
Meat						
Beef, N (%)	24.0 (92.3)	10.0 (38.5)	0	9.0 (34.6)	4.0 (15.4)	1.0 (3.8)
Chicken, N (%)	26.0 (100.0)	11.0 (42.3)	0	6.0 (23.1)	8.0 (30.8)	1.0 (3.8)
Fish, N (%)	14.0 (53.9)	3.0 (11.5)	0	4.0 (15.4)	2.0 (7.7)	5.0 (19.2)
Sausage, N (%)	20.0 (76.9)	1.0 (3.8)	0	12.0 (46.2)	2.0 (7.7)	5.0 (19.2)
Egg, N (%)	25.0 (96.1)	8.0 (30.8)	0	12.0 (46.2)	4.0 (15.4)	1.0 (3.8)
Ham/Salami/Mortadella, N (%)	12.0 (46.2)	2.0 (7.7)	0	5.0 (19.2)	2.0 (7.7)	3.0 (11.5)
Drinks						
Sugar-free coffee, N (%)	2.0 (7.7)	2.0 (7.7)	0	0	0	0
Coffee with sugar, N (%)	22.0 (84.6)	15.0 (57.7)	7.0 (26.9)	0	0	0
Sweetened coffee, N (%)	5.0 (19.2)	3.0 (11.5)	1.0 (3.8)	0	0	1.0 (3.8)
Beer, N (%)	2.0 (7.7)	0	0	1.0 (3.8)	0	1.0 (3.8)
Artificial juice, N (%)	10.0 (38.5)	5.0 (19.2)	1.0 (3.8)	1.0 (3.8)	0	3.0 (11.5)
Soda, N (%)	15.0 (57.7)	4.0 (15.4)	0	6.0 (23.1)	1.0 (3.8)	4.0 (15.4)

6 DISCUSSION

This study aimed to evaluate the effect of creatine supplementation on muscle mass, strength, and functional capacity of older people with stroke. In addition, biochemical markers of inflammation, apoptosis, mitochondrial function, muscle degradation, and synthesis were assessed. The study involved the participation of older people with moderate ischemic stroke who were randomized into two groups presenting similar aspects regarding the demographic and clinical data, severity, previous comorbidities, and nutritional risk at the baseline. During hospitalization, clinical treatment, caloric and protein intake, and physical therapy received were similar for both groups. It was a software-randomized, double-blind, controlled exploratory clinical trial in a stroke Unit. Food intake at each meal was closely assisted, with protein requirement individually calculated according to daily food intake. All participants were evaluated in the first seven days of hospitalization due to stroke, a moment with a lack of studies on nutrition, food intake, and supplementation with particular nutrients.

In this study, one group received creatine, and the other received a placebo. In the group participants treated with creatine, an increase in serum creatinine values was observed, indicating that, even in a short period of intervention, there was a biological effect. Around 2% of endogenous creatine is excreted daily as creatinine, and the increase in serum creatinine reflects an increase in ingested creatine. (73) In fact, with all the difficulties common to patients after stroke concerning dysphagia and lack of appetite, patients readily accepted any supplement offered, be it creatine, placebo, or whey protein. Additionally, creatine supplementation proved safe considering the observed adverse events, most of which were not treatment-related.

Despite the methodological rigor adopted, this study had its limitations, mainly because it is a small clinical trial, which can be helpful as a pilot for future work. Another significant limitation is the condition of the patient after the stroke, as these individuals often present several aspects that can disrupt food intake, such as anorexia aggravated by the inflammatory process in the initial phase of the stroke, lowered level of consciousness, communication difficulties, dysphagia and frequent periods of fasting for tests and procedures. Additionally, these individuals present immobility or reduced mobility in the initial phase, thus making it difficult for the early mobility training protocol to be more intensified, as it happens in studies with creatine supplementation in other populations.

However, it was decided to develop a small clinical trial of the exploratory type to maintain methodological rigor and greater control of the analyzed variables within the acute conditions after the stroke. In addition, other studies with creatine supplementation were developed with similar numbers of participants, showing some positive findings. (41)

In fact, the interest of this study was to assess whether creatine would be effective in being used in the earliest phase of stroke. This is because establishing nutritional therapy, at this moment, right after the stroke, characterised by the metabolic response to stress, is essential to prevent future losses of muscle mass and strength. (74) In the OPENS trial, it was observed that the group allocated to receive a hypocaloric diet had higher mortality in 90 days. The Kaplan-Meier curve exhibited an early separation, showing the importance of nutritional supplementation in the acute phase. There are, however, gaps in the literature regarding general nutritional needs and whether they would benefit from receiving any specific compound.

In this sense, creatine was chosen for its potential benefits in situations with ischemia, acting as an additional energy source and stimulating protein synthesis pathways, among others. Some experimental evidence shows that pre or post-treatment with creatine can decrease the damage induced by ischemia in both the heart and brain. (75)

The Creatine (Cr)–Phosphocreatine (PCr) system is of major relevance in tissues with high and unsteady energy requirements, such as muscle, heart, and brain, because it provides a ready-release high-energy phosphate pool for maintaining adequate ATP levels. Inside the cells, the creatine kinase (CK) enzyme reversibly converts Cr into PCr. Under ATP exhaustion (such as ischemia), PCr can donate its phosphate group to the ADP to transform it into ATP, even without oxygen and glucose. (76)

Therefore, although we knew that the exposure to physical activity would not be intense and the intervention time was short, we chose to offer a higher dose of creatine to look for effect. The dose of approximately 10 g twice a day has already been studied by Gotshalk et al. In active older men, the seven days supplementation was effective at increasing several indices of muscle performance, including functional tests without adverse side effects. (40)

In a review published by Candow et al. in 2021, the authors cite the potential benefits of creatine for maintaining muscle health but warn of the lack of studies on osteosarcopenia,

sarcopenic obesity, cachexia and Frailty Syndrome. Likewise, we did not find studies that evaluated creatine supplementation in the acute phase of stroke. (73)

Considering the main objective, it was observed that creatine supplementation did not influence body weight, muscle strength, functional capacity, and measurements indicative of muscle mass evaluated by the thickness of the rectus femoris and biceps muscles by ultrasound, as well as by the evaluation of fat-free mass assessed by bioimpedance. However, there was an increase in AMMI in men but not in women. Indeed, the muscular response to creatine is different in men and women, as already observed in previous studies. The reason is that perhaps women already have enough muscle creatine responding less to supplementation. Additionally, most studies with positive creatine effects are found in men. (77,78) However, this acute increase in muscle mass may be due to muscle edema since creatine transport into the cell depends on sodium. Other authors believe that cellular edema and muscular creatine uptake can trigger the stimulus for protein synthesis. (79) The finding of our study makes sense since the other bioimpedance parameters did not change with creatine supplementation, showing a localized effect on the appendicular muscles.

As for the fact that creatine does not improve strength and functional capacity, this effect may be negative or a consequence of the short intervention period and or the impossibility of practising more intense exercises offered in early mobility training sessions.

The acute phase of stroke differs from the conditions in which the majority of studies with creatine supplementation are performed, which are outpatient studies that involve physical exercise in states of adequate health or more chronic conditions such as ageing. (41,73) The acute phase of stroke is marked by the metabolic response to stress characterised by hypercatabolism and hypermetabolism. In addition, it is observed that after acute injury, there is innate immune activation leading to systemic inflammation, associated with hormonal changes such as increased catecholamines, reduced hormones such as IGF-1, and insulin resistance, which lead to lipolysis and protein catabolism to generate substrates that will help resolve the injury. (80,81) In fact, after the stroke, there was an increase in loss of muscle mass and function by 10% after ten days of the event and 20% after one month; (82) therefore, strategies to counteract this loss are required.

Thus, from a mechanistic perspective, inflammatory and hormonal markers change before phenotypic and functional changes may be observed. From the perspective of stimulating protein synthesis and degradation, the dosage of IGF-1, IL-6, insulin resistance,

and P3NP and 3-MH were performed. IGF-1 is known to stimulate protein synthesis; however, IL-6, as previously discussed, has a paradoxical role. The muscles secrete IL-6, essential for protein synthesis and muscle regeneration. (62,63) At the same time, IL-6 is an acute phase protein that has its values increased in the initial phase after stroke and its values are expected to decrease with the passage of time and resolution of the inflammatory process. (61,83). The paradox exists about acute increases after muscle injury with the participation of IL-6 in an anti-inflammatory and regenerative way versus chronic and not-so-high elevation associated with pro-inflammatory and catabolic action. (62,63) In the present study, there was no statistically significant difference regarding the use of creatine and serum levels of IL-6 and IGF-1. However, plotting the graph of the means of the groups between the two moments follows the same direction. Therefore, other studies with more individuals may contribute to elucidating the role of creatine in stimulating the acute elevation of IL-6 and IGF-1, both potentially related to increased protein synthesis. (78)

During the conversion of procollagen to collagen, the P3NP propeptide of procollagen is released into circulation as an early anabolic response to testosterone. Therefore, we decided to evaluate P3NP as a potential protein synthesis marker. However, there were no differences between the groups. (27) Insulin resistance was also studied by measuring insulin and glucose and calculating the HOMA-IR; however, creatine did not influence this parameter. (78)

On the other hand, the markers of muscle degradation evaluated by 3-MH, a metabolite of the breakdown of contractile proteins and is a marker of muscle degeneration, were also not statistically significant. However, in the same way, graphic design is directed towards increased degradation in the creatine group. This evaluation was carried out by 2 methods, one with labeled isotope and serial serum dosage that allows evaluating the percentage of protein degradation and serum level in an isolated sample and performed by ELISA. Both methods show similar graphic figures, reinforcing the veracity of the assessment. As observed in a previous study, there was a slight increase in 3-MH during creatine supplementation and no increase in leucine oxidation in women. (77,78) Since in men, there was leucine oxidation and 3-MH reduction. Thus, when sex is added to the labeled isotope model, it is observed that there is an interaction between treatment and moment, but not with sex. However, the graphic design once again points to the direction of women being more subject to degradation than men. This method, being more complex, was performed on five

patients from each group, and therefore, due to the small sample size, it only allows us to discuss possibilities in this regard. However, it is worth remembering that due to the complexity of the evaluation in general, studies with labeled isotopes and dosage with liquid chromatography-mass spectrometry commonly study few patients, with studies with 4 individuals per group being found. By adding sex in the models of IGF-1, IL-6, and 3-MH in a sample isolated by ELISA, there were no changes in the patterns.

Regarding inflammation, several acute and chronic models in vitro, in animals and humans, show potential benefits of using creatine in reducing inflammatory mediators such as factor nuclear kappa B, Tumour necrosis factor α (TNF- α), and C-reactive protein, among others. In the present study, we chose to study progranulin and GDF-15.

Progranulin is a glycoprotein that has been studied in recent years. It has important bodily functions, such as involvement in cell proliferation and tissue repair, and has an essential role as an anti-inflammatory and neuroprotective factor. (25,84) This compound binds to the TNF- α receptor, competing with it, and exerts an anti-inflammatory effect. On the other hand, as a tissue growth factor, it is associated with tumorigenesis, with high levels in patients with breast, ovarian, gastric, and other cancers. (85)

Acute cerebral ischemia triggers a local and systemic immune response proportional to the lesion's size. Typically, progranulin is reduced in this context, favoring the inflammatory process and muscle degeneration. (28) Studies in experimental animals have shown that progranulin has beneficial effects in acute cerebral ischemia, acting on the regeneration of injured peripheral neurons, protecting the blood-brain barrier, activating microglia, and promoting the reduction of inflammation and edema in the tissue brain, in addition to acting on the vascular endothelium causing vasodilation that has a positive effect on the region affected by ischemia. (86,87)

On the other hand, studies with humans in the acute phase of stroke showed that this marker was increased in these individuals. Those with a more severe stroke and significant functional disability had even higher progranulin levels. In these studies, the researchers observed an association between increased progranulin and mortality and worse functional prognosis in this population. (28,29) Likewise, higher serum levels of progranulin are markers of worse prognosis in cancer patients. (85) The protective effects observed in experimental studies in acute cerebral ischemia do not seem to be confirmed when observing the behaviours of progranulin in humans who have suffered a stroke. In our findings, a reduction

in progranulin was observed in both groups; however, in the group that received creatine, but not in the control group this reduction was pronounced. This results represents the anti-inflammatory role of creatine, but we cannot state the real benefit of this finding in the long term. It is observed that the average of progranulin after the seven days of creatine administration was within limits previously established by Xie et al. (29) Progranulin emerges as a potential target for new therapies, with its administration being studied in chronic inflammatory diseases such as arthritis and in cases of dementia. On the other hand, anti-progranulin antibodies (anti-PGRN monoclonal antibody A23) are being studied in some tumours. (85) Thus, the novelty of this study is to show the potential of creatine to modulate progranulin in the acute phase of the stroke - paving the way for new studies to identify the role of progranulin in the prognosis of stroke and if modulating its serum levels could bring some benefit.

GDF-15, also called myomitokine, is secreted after stress response to an injury. GDF15 has been extensively studied as a biomarker for various diseases, including chronic inflammation, cancer, musculoskeletal, cardiovascular, kidney, liver, and neurological diseases. (88) Moreover, it is a biomarker of apoptosis and mitochondrial dysfunction. It is associated with low muscle mass and a worse prognosis for diseases. In the present study, there was a difference between the moments, but without the influence of creatine, reinforcing its role in being secreted in acute situations.

Regarding the 90-day follow-up, it showed that there are no differences between mortality, and there is a recovery of functional capacity and strength on the unaffected side, showing the safety of the intervention, but without the participation of creatine.

In our study, we evaluated the perception of quality of life 90 days after the stroke and no differences were observed between the treatment and control groups. Results related to the quality of life are generally associated with the individual's degree of disability. Studies have shown that patients who had a post-discharge rehabilitation plan had a better perception of quality of life and this perception has an impact on the degree of disability after the ictus. (89–91)

Finally, a secondary aspect observed in this study, not related to creatine supplementation but which brings essential information, is about aspects related to the nutritional therapy offered to all patients in the study. The nutritional risk assessed by the NRS 2002 at the time of hospital admission showed that 56.7% of the patients included in the study

were at risk of developing a problem related to nutritional status. Considering the complications after a stroke that culminate in the development of malnutrition and disease-related muscle mass, it is imperative to screen these patients as soon as they are admitted to the hospital. Afterwards, it is necessary to make a dietary plan to prevent this patient from worsening in the parameters that force muscle, loss of mass and function. (12,15)

Therefore, it is observed that there is potential for creatine to modulate some aspects related to inflammatory biomarkers in acute stroke. Its potential to regulate protein synthesis and degradation in this phase is still being determined. Females behave differently in their response regarding muscle mass and protein degradation when compared to males. Additionally, the creatine administration proved to be easy to accept and safe. The caloric supply of 21-25 kcal/kg with 1.5g of protein/kg per day proved adequate for weight maintenance regardless of creatine administration.

In summary, creatine did not impact functional capacity, and strength but increased appendicular muscle mass in men. In fact, women and men respond differently to creatine supplementation. Hormones and inflammatory mediators (IGF-1 and IL-6) potentially related to protein synthesis tended to increase with creatine supplementation. However, the percentage of muscle breakdown increased in the creatine group when sex was added to the model. However, it is impossible to know if there was indeed protein degradation or if this happened proportionally to the increase in protein synthesis.

Our results provided important information about inflammatory biomarkers, such as progranulin, which was reduced over time and with creatine supplementation. It was observed that GDF-15 is an inflammatory marker that decreases over time, regardless of the intervention. The work brought essential data related to caloric-protein intake during hospitalization, showing the importance of nutritional adjustment to avoid weight loss during this period. Supplementation proved to be safe from the point of view of adverse events occurring in the period; the participants had good tolerance and good acceptance. Therefore, studies with a larger number of participants and perhaps a longer intervention time can be carried out to fill this gap in the literature regarding creatine supplementation in the acute phase of stroke. This study functioned as a pilot and may, contribute to the calculation of sample size, choice of variables, and measurement standards so that new research that evaluates creatine supplementation after stroke is developed. Finally, the role of progranulin

in the acute phase of stroke and its reduction with the use of creatine need to be explored in future studies, as this is a site of therapeutic potential.

7 CONCLUSION

Creatine supplementation proved to be safe, with good tolerance and easy acceptance. Creatine did not impact functional capacity, and strength but increased appendicular muscle mass in men. It has the potential to modulate some inflammatory markers after stroke and had an influence on the reduction of progranulin values, reflecting its anti-inflammatory role. Supplementation had no impact on mortality and quality of life after the event. This study helped to understand the potential role and safe of creatine in the acute phase of the stroke, which supports the development of other trials. In fact, studies with larger number of participants and longer intervention time should be carried out to better understand the influence of creatine supplementation in the acute phase of stroke.

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APPENDIX A – Informed Consent Form



Universidade Estadual Paulista "Júlio de Mesquita Filho"
Faculdade de Medicina de Botucatu Departamento
de Clínica Médica



Termo de Consentimento Livre e Esclarecido

O(a) senhor(a) está sendo convidado a participar de uma pesquisa chamada **"Influência da suplementação de proteína e creatina capacidade funcional, qualidade de vida e função muscular após acidente vascular cerebral"**, que pretende verificar se o consumo de proteína isolada do soro do leite + creatina associado à fisioterapia durante 7 dias promovem melhor: capacidade funcional e da qualidade de vida de homens idosos que sofreram AVC após 90 dias da alta hospitalar, além de ganho de massa, força e função muscular, melhora do perfil inflamatório e resistência insulínica no período de internação

O(a) senhor(a) foi selecionado para participar dessa pesquisa por estar entre os pacientes internados na unidade de acidente vascular cerebral do Hospital das Clínicas da Unesp de Botucatu. A pesquisa consta de 3 dias específicos de avaliações e testes (Nas primeiras 24 horas de internação, no sétimo dia de internação e 90 dias após a alta hospitalar durante consulta médica no ambulatório) conforme descritos abaixo:

- 1) Uma avaliação nutricional com protocolo de atendimento específico, realização de avaliação física (medidas de braço, perna, peso e altura), exame de composição do corpo (impedância bioelétrica e ultrassom muscular) e exame de força (dinamometria). Os procedimentos têm por finalidade conhecer o perfil dos pacientes internados e fazer o acompanhamento dos mesmos logo quando dão entrada na unidade até o momento da alta hospitalar;
- 2) O(a) senhor(a) irá receber 2 sachês ao dia de suplemento alimentar (proteína + creatina) ou (proteína + placebo) durante 7 dias da internação na unidade de AVC. O suplemento deverá ser tomado da seguinte forma: Diluir 1 sachê em 300ml de água em temperatura ambiente e consumir após café da manhã e 1 sachê após jantar. O preparo do suplemento será feito pela equipe de enfermagem da unidade. O consumo do suplemento

pode causar um pequeno desconforto abdominal e se caso isto vier a ocorrer, comunicar o pesquisador para que as providências sejam tomadas;

3) Durante a internação na unidade, o senhor irá receber o atendimento de fisioterapia padrão individualizado e direcionado para suas necessidades, assim como qualquer outro paciente não participante do estudo;

4) Serão feitas duas coletas de sangue (a primeira no primeiro dia de internação e a segunda no sétimo dia de internação). O(a) senhor(a) será orientado quanto à coleta de sangue e quanto aos exames que serão avaliados. Estes exames servem para verificar como está o perfil de inflamação após o AVC e serão pedidos em 2 momentos para verificar se com o consumo do suplemento alimentar ocorreu melhora nos valores dos exames. A coleta de sangue pode levar a alguns desconfortos, como: dor e hematoma no local da picada e raramente tontura ou desmaios.

5) Para avaliar se houve perda de músculo durante os 7 dias de internação, será feita a avaliação da degradação muscular utilizando a 3-metilhistidina. Para tal exame serão feitos os seguintes procedimentos nas primeiras 24 horas de internação e após 7 dias de internação: a) O(a) senhor(a) irá tomar 50mL de um líquido contendo o marcador (substância que quando consumida aparece no exame de sangue para verificar se houve diminuição do músculo), 18 horas após ingerir o líquido com marcador, serão feitas 3 coletas de sangue (10ml cada coleta) com intervalo de 1 hora entre elas.

6) Os resultados dos exames de sangue realizados na rotina da unidade também serão utilizados para a pesquisa, além de pesquisa de dados gerais no prontuário eletrônico.

Com este estudo vamos poder verificar se o consumo de suplemento alimentar associado ao tratamento de fisioterapia irá trazer benefícios aos pacientes que sofreram AVC. É importante ressaltar que tanto a participação quanto a desistência da participação na pesquisa não irão influenciar no agendamento/atendimento médico ou de qualquer especialidade que venha precisar. O(a) senhor(a) poderá retirar seu consentimento em qualquer fase da pesquisa sem nenhum prejuízo. É garantido total sigilo do seu nome, imagem, resultados de exames e diagnósticos de doenças em relação aos dados relatados nesta pesquisa.

Para o caso do paciente convocado a participar da pesquisa não ter condições físicas ou psicológicas de concordar/discordar de sua participação ou assinar o termo de consentimento livre e

esclarecido, torna-se possível o consentimento e assinatura de um responsável próximo para tal. O(a) senhor(a) receberá uma via deste termo, e outra via será mantida em arquivo pelo pesquisador por cinco anos.

Qualquer dúvida adicional, você poderá entrar em contato com o Comitê de Ética em pesquisa, através do fone (14) 3880-1608 / 1609. Endereço Chacára Butignoli s/n, Rubião Júnior - Botucatu - São Paulo CEP: 18618-970 (Próximo a FAMESP). E-mail: cep@fmb.unesp.br Horário de atendimento: de segunda a sexta, das 8hs às 11h30min e das 13h às 17h30min.

CONCORDO EM PARTICIPAR DA PESQUISA

Nome do paciente: _____ Data: ____/____/____

Assinatura do paciente: _____

Nome do responsável pelo paciente: _____

Assinatura do responsável pelo paciente: _____

Agradecemos a sua colaboração e ficamos à disposição para maiores esclarecimentos.

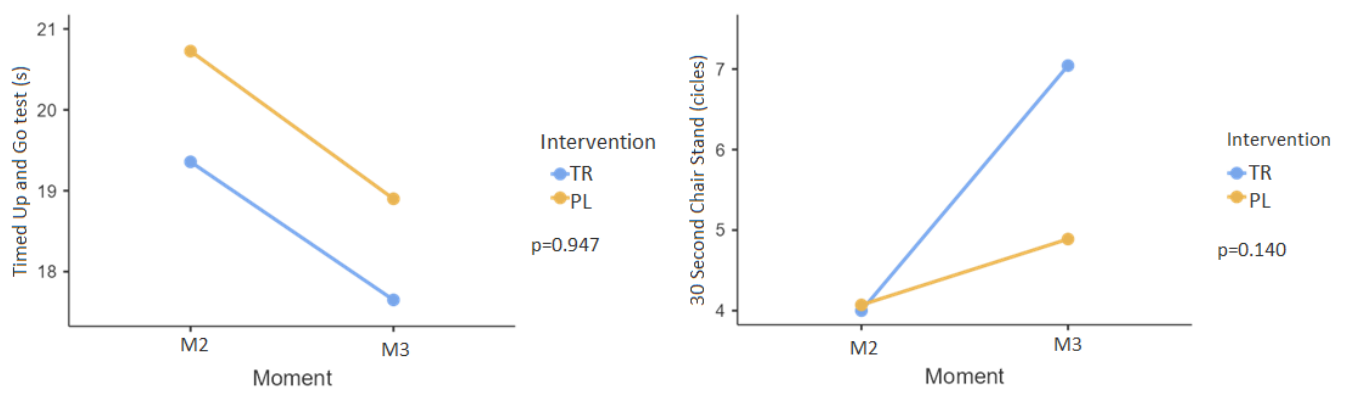
Juli Thomaz de Souza

Aluna de pós-graduação: Juli Thomaz de Souza
Faculdade de Medicina de Botucatu – Dep. de Clínica Médica, Tel: (14) 3811-6213/
Celular e WhatsApp: (19) 98423-0151. E-mail: jtsouz@yahoo.com.br

Orientadora: Profª. Drª. Paula Schmidt Azevedo Gaiolla
Faculdade de Medicina de Botucatu - Depto de clínica Médica, fone (14) 3811-6213.
E-mail: schmidt.azevedo@unesp.br

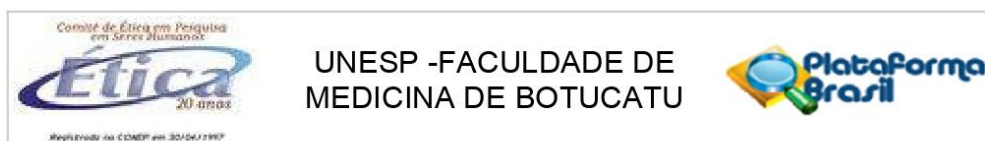
APPENDIX B – RESULTS OF MUSCLE STRENGTH AND FUNCTION AFTER 90 DAYS

Figure – Funcional capacity and muscle strenght evaluated by Timed Up and Go test and 30 second Chair Stand test of older people who participated in the ICaRUS Stroke Trial in the 7th day of hospitalization and on the 90 days after stroke with creatine versus placebo



Source: material prepared by the author. **TR:** Treatment; **PL:** Placebo; **M2:** Moment 2 (7th day of hospitalization); **M3:** Moment 3 (90 days after stroke).

ANNEX A - Approval document of Research Ethics Committee from Botucatu Medical School



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INFLUÊNCIA DA SUPLEMENTAÇÃO DE PROTEÍNA E CREATINA NA CAPACIDADE FUNCIONAL, QUALIDADE DE VIDA E FUNÇÃO MUSCULAR APÓS ACIDENTE VASCULAR CEREBRAL

Pesquisador: JULI THOMAZ DE SOUZA

Área Temática:

Versão: 1

CAAE: 79844317.3.0000.5411

Instituição Proponente: Departamento de Clínica Médica

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.421.189

Apresentação do Projeto:

O Acidente Vascular Cerebral é definido como uma disfunção neurológica aguda de origem vascular, com desenvolvimento súbito de sinais clínicos de distúrbios da função cerebral com duração de mais de 24 horas.

Após o evento, os pacientes em geral ficam mais suscetíveis às perdas de peso, ocorrendo com frequência redução do apetite, disfagia, depressão e alterações da mobilidade, com consequente dependência funcional.

Adicionalmente, existe a possibilidade da redução da massa muscular e desenvolvimento de sarcopenia representando um fator de risco poderoso para a fragilidade.

No contexto do AVC, é comum observar inatividade física, alterações inflamatórias, alterações neurohumorais e carência nutricional de macro e micronutrientes. Indivíduos idosos apresentam maior necessidade proteica e estudos mostram que a suplementação com proteína e creatina pode melhorar a sua massa muscular. No entanto, a ação da suplementação de nutrientes específicos em indivíduos que sofreram AVC ainda é pouco conhecida e os estudos escassos.

Este estudo é um ensaio clínico randomizado e duplo-cego onde serão incluídos 60 homens idosos com AVC desde a fase aguda até 6 semanas após alta.

Eles receberão suplementação com proteína + creatina ou somente proteína e passarão por protocolo de atendimento em fisioterapia.

Endereço: Chácara Butignolli, s/n

Bairro: Rubião Junior

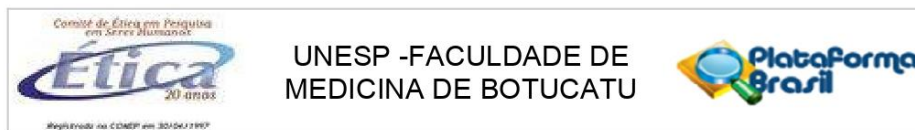
UF: SP

Município: BOTUCATU

Telefone: (14)3880-1609

CEP: 18.618-970

E-mail: cep@fmb.unesp.br



Continuação do Parecer: 2.421.189

Ao final da intervenção o objetivo será avaliar se a suplementação associada à fisioterapia promoveu melhora da capacidade funcional, força, função muscular e qualidade de vida nesta população. Serão avaliados 60 homens idosos que internarem com diagnóstico de AVC na Unidade de Acidente Vascular Cerebral do Hospital das Clínicas da Faculdade de Medicina da UNESP de Botucatu. Os pacientes serão divididos em 2 grupos (CONTROLE/ESTUDO). Grupo de estudo receberá suplementação com 30g de proteína isolada do soro do leite + 2,5g de creatina + atendimento de fisioterapia e o grupo controle 30g de proteína isolada do soro do leite + atendimento de fisioterapia. A intervenção terá duração de 6 semanas com início nas 72 primeiras horas após AVC. As avaliações serão realizadas da seguinte forma: Momento 1 (M1) – Primeiras 72h de internação (Unidade de AVC) – Avaliação de composição corporal, avaliação de força muscular por força de preensão manual, avaliação antropométrica, coleta de sangue e randomização. Momento 2 (M2) – Alta hospitalar (Unidade de AVC) – Avaliação de composição corporal, avaliação de força muscular por força de preensão manual, avaliação da função muscular por teste de caminhada e avaliação antropométrica. Momento 3 (M3) – 6ª semana (Setor de Reabilitação) – Realização das avaliações antropométrica, de composição corporal, força muscular por força de preensão manual e força e função de membros inferiores, capacidade funcional, qualidade de vida, do hábito alimentar e também coleta de sangue para dosagens bioquímicas.

Objetivo da Pesquisa:

Objetivo Primário: Verificar se o consumo de proteína isolada do soro do leite + creatina associado à fisioterapia duas vezes por semana promovem melhor capacidade funcional e da qualidade de vida de homens idosos que sofreram AVC com 6 semanas de acompanhamento.

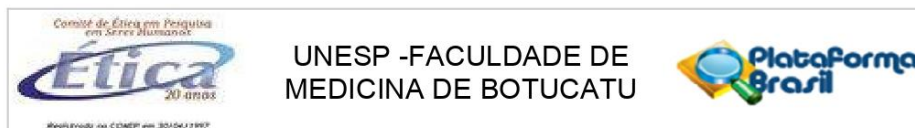
Objetivo Secundário: Verificar se o consumo de proteína isolada do soro do leite + creatina associado à fisioterapia duas vezes por semana promovem melhor ganho de massa, força e função 1 e 6 semanas após o AVC; verificar melhora do perfil inflamatório e resistência insulínica 1 e 6 semanas após o AVC e verificar as mudanças da composição corporal, força muscular, mediadores inflamatórios e resistência insulínica nas primeiras 72 horas de internação, 1 semana e 6 semanas após AVC.

Avaliação dos Riscos e Benefícios:

Segundo os autores, os riscos se relacionam à eventual intolerância a algum componente do suplemento causando desconforto abdominal.

Benefício individuais poderão ocorrer, como o ganho de massa muscular e melhora do estado nutricional acarretando em aumento da capacidade funcional após AVC

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Telefone: (14)3880-1609	E-mail: cep@fmb.unesp.br



Continuação do Parecer: 2.421.189

Comentários e Considerações sobre a Pesquisa:

O Projeto apresenta boa justificativa para sua realização, trazendo objetivos e metodologia claros.
As intervenções propostas parecem ser benéficas e os riscos estão esclarecidos no TCLE.
Os critérios de inclusão e exclusão estão bem definidos.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos e declarações foram anexados, incluindo o referente ao biorrepositório.
O TCLE é claro, esclarece os riscos e de fácil compreensão.

Recomendações:

Não se aplicam.

Conclusões ou Pendências e Lista de Inadequações:

Sou de parecer favorável à aprovação do projeto, sem necessidade de envio à CONEP.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado em reunião ordinária do Comitê de Ética em Pesquisa da FMB/UNESP, realizada em 04 dezembro de 2017, o projeto encontra-se APROVADO, sem necessidade de envio à CONEP.

O Comitê de Ética em Pesquisa, no entanto, informa que ao final da execução da pesquisa, seja enviado o "Relatório Final de Atividades", na forma de "Notificação", via Plataforma Brasil.

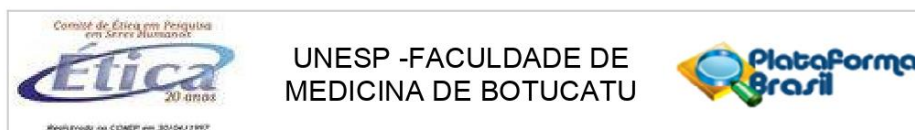
Atenciosamente,

Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu - UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1012695.pdf	11/11/2017 13:08:22		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEdoutorado.pdf	11/11/2017 13:07:50	JULI THOMAZ DE SOUZA	Aceito

Endereço: Chácara Butignolli, s/n
Bairro: Rubião Junior CEP: 18.618-970
UF: SP Município: BOTUCATU
Telefone: (14)3880-1609 E-mail: cep@fmb.unesp.br



Continuação do Parecer: 2.421.189

Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	biorrepositorioJuli.pdf	11/11/2017 13:05:49	JULI THOMAZ DE SOUZA	Aceito
Outros	TermoDeAnuencialInstitucional.pdf	06/11/2017 09:11:58	JULI THOMAZ DE SOUZA	Aceito
Folha de Rosto	FolhaDeRosto.Pdf	06/11/2017 09:10:16	JULI THOMAZ DE SOUZA	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoAVC.pdf	06/11/2017 09:06:19	JULI THOMAZ DE SOUZA	Aceito
Outros	AnuenciaHcfmb.pdf	06/11/2017 09:00:50	JULI THOMAZ DE SOUZA	Aceito
Cronograma	CronogramaProjeto.pdf	06/11/2017 08:59:12	JULI THOMAZ DE SOUZA	Aceito
Declaração de Instituição e Infraestrutura	AnaliseDgaaHcfmb.pdf	06/11/2017 08:51:10	JULI THOMAZ DE SOUZA	Aceito
Orçamento	PlanilhaOrcamentariaUpeclin.Pdf	06/11/2017 08:50:05	JULI THOMAZ DE SOUZA	Aceito
Declaração de Instituição e Infraestrutura	AceiteUnipex.Pdf	06/11/2017 08:49:37	JULI THOMAZ DE SOUZA	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

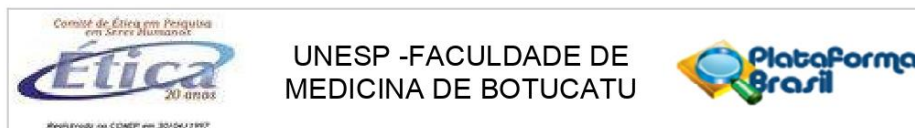
Não

BOTUCATU, 07 de Dezembro de 2017

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador)

Endereço: Chácara Butignolli, s/n
Bairro: Rubião Junior
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Telefone: (14)3880-1609 **CEP:** 18.618-970
E-mail: cep@fmb.unesp.br

**ANNEX B - Approval document of Research Ethics Committee from Botucatu Medical School
- Amendment 1**



PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: INFLUÊNCIA DA SUPLEMENTAÇÃO DE PROTEÍNA E CREATINA NA CAPACIDADE FUNCIONAL, QUALIDADE DE VIDA E FUNÇÃO MUSCULAR APÓS ACIDENTE VASCULAR CEREBRAL

Pesquisador: JULI THOMAZ DE SOUZA

Área Temática:

Versão: 2

CAAE: 79844317.3.0000.5411

Instituição Proponente: Departamento de Clínica Médica

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.246.038

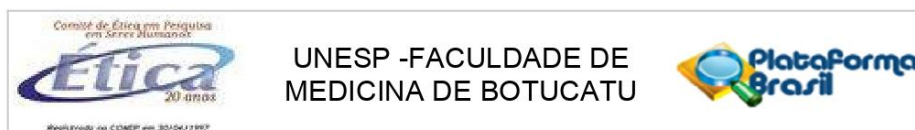
Apresentação do Projeto:

As informações descritas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas dos documentos e arquivo - Informações Básicas da Pesquisa.

O acidente vascular cerebral (AVC) é uma disfunção neurológica aguda de origem vascular, com desenvolvimento súbito de sinais clínicos de distúrbios da função cerebral com duração de mais de 24 horas. É a principal causa de incapacidade na vida adulta e os pacientes nesta fase tornam-se, em geral, mais suscetíveis às perdas de peso involuntárias, ocorrendo com frequência redução do apetite, disfagia, depressão e alterações da mobilidade, com consequente dependência funcional, além da possibilidade da redução da massa muscular e desenvolvimento de sarcopenia representando um fator de risco poderoso para a fragilidade. Durante o período de internação, logo após o AVC, intensifica-se a inatividade física, alterações inflamatórias, alterações neuro-humorais e carência nutricional, que já são comuns em todo período tardio pós o evento. Indivíduos idosos apresentam maior necessidade proteica e a suplementação com proteína e creatina pode melhorar a sua massa muscular. No entanto, a ação da suplementação de nutrientes específicos em indivíduos que com AVC durante a internação ainda é pouco conhecida.

O objetivo será avaliar se a suplementação de proteína e creatina durante a internação promove melhora da capacidade funcional e qualidade de vida após 90 dias do AVC. Adicionalmente, avaliar

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Continuação do Parecer: 3.246.038

os mecanismos envolvidos na degradação muscular durante o período de internação.

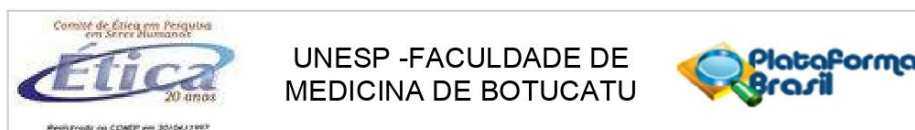
Metodologia: Trata-se de ensaio clínico randomizado e duplo-cego no qual serão incluídos 60 homens idosos durante internação por AVC. Os pacientes serão divididos em dois grupos: Proteína+ Creatina (PC): receberão suplementação com proteína, em dose já recomendada pela literatura + creatina ou Proteína + Placebo (PP): receberão proteína, em dose já recomendada pela literatura + placebo. Os dois grupos terão atendimento de fisioterapia convencional. Nas primeiras 24h e no sétimo dia serão avaliados degradação muscular por meio da dosagem de 3-metil-histidina e composição corporal por água marcada pela técnica de isótopos marcados. Serão avaliadas dosagens hormonais de insulina e IGF-1 e dosagem de marcadores inflamatórios como interleucina-6 e metaloproteases. Para a comparação entre as variáveis e da variação das variáveis durante os 7 dias, serão utilizados o teste t de Student se as variáveis apresentarem distribuição normal ou teste Mann-Whitney se apresentarem distribuição não normal. A análise de fatores associados a capacidade funcional será realizada por regressão logística múltipla. Nível de significância 5%. Trata-se de emenda que propõe alteração do tempo de intervenção, da dose do suplemento ofertada e também complementação da avaliação dos pacientes. Justificativa da emenda: Estas alterações foram necessárias para tornar o estudo mais factível e adequado ao que é encontrado na literatura.

Objetivo da Pesquisa:

Objetivo Primário: Verificar se a suplementação com proteína isolada do soro do leite + creatina associada à fisioterapia convencional durante 7 dias de internação promove melhora da capacidade funcional e da qualidade de vida 90 dias após AVC. **Emenda 1:** Verificar se a suplementação com proteína isolada do soro do leite + creatina associada à fisioterapia convencional durante 7 dias de internação promove melhora da capacidade funcional e da qualidade de vida 90 dias após AVC.

Objetivo Secundário: Verificar se a suplementação com proteína isolada do soro do leite + creatina associada à fisioterapia convencional durante 7 dias de internação reduz perda da massa muscular, perfil inflamatório e resistência insulínica durante internação por AVC. **Emenda 1:** Verificar se a suplementação com proteína isolada do soro do leite + creatina associada à fisioterapia convencional durante 7 dias de internação reduz perda da massa muscular, perfil inflamatório e resistência insulínica durante internação por AVC.

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Continuação do Parecer: 3.246.038

Avaliação dos Riscos e Benefícios:

Riscos: Intolerância a algum componente do suplemento causando desconforto abdominal.

Benefícios: Ganho de massa muscular e melhora do estado nutricional acarretando em aumento da capacidade funcional após AVC.

Comentários e Considerações sobre a Pesquisa:

Trata-se de emenda para realização de alteração do tempo de intervenção, da dose do suplemento ofertada e também complementação da avaliação clínica com a aplicação do questionário: "National Institute of Health Stroke Scale" – NIHSS (momentos 1 e 2 da metodologia); da Avaliação da degradação muscular (com utilização do marcador D3-Metil histidina) no momento 2; e acrescentado o Momento 3 (M3) - 90 dias após AVC (Ambulatório de Doenças Neurovasculares), com as seguintes avaliações: Avaliação clínica (aplicação da escala de NIHSS); Avaliação do grau de dependência para atividades diárias (Índice de Barthel); Avaliação da capacidade funcional (Teste de caminhada de 6 min; teste Timed Up and Go; Escala de Rankin modificada (eRm)); Avaliação de força muscular (Força de preensão manual - Handgrip; teste de Fullerton); Avaliação dos hábitos alimentares (Recordatório de 24 horas; questionário de frequência alimentar); Avaliação da qualidade de vida (aplicação do The European 5D Quality of Life Scale - EuroQol-5D). Justificativa da emenda apresentada pelos pesquisadores: Estas alterações foram necessárias para tornar o estudo mais factível e adequado ao que é encontrado na literatura.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados todos os documentos necessários, incluindo TCLE readequado, em forma de convite e objetivo, conforme Resolução 466/2012.

Recomendações:

Apresentar relatório final de atividades após conclusão da pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

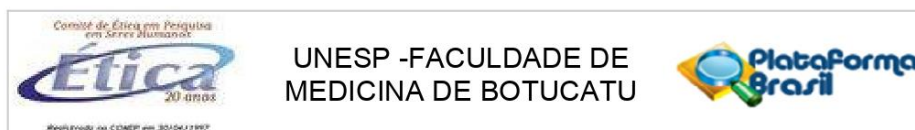
Após análise em REUNIÃO ORDINÁRIA, o Colegiado deliberou APROVADA a emenda apresentada.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado em REUNIÃO ORDINÁRIA do Comitê de Ética em Pesquisa da FMB/UNESP, realizada em 1º de ABRIL de 2019, a EMENDA apresentada, encontra-se APROVADA, sem necessidade de envio à CONEP.

Atenciosamente,

Endereço: Chácara Butignolli, s/n	CEP: 18.618-970
Bairro: Rubião Junior	
UF: SP	Município: BOTUCATU
Telefone: (14)3880-1609	E-mail: cep@fmb.unesp.br



Continuação do Parecer: 3.246.038

Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu – UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

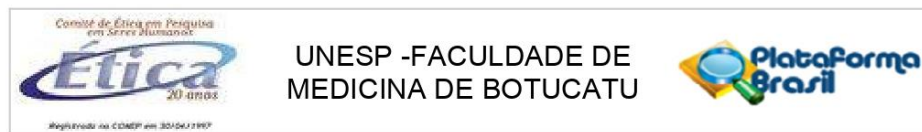
Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1310819_E1.pdf	20/03/2019 16:37:49		Aceito
Outros	Emenda_1.pdf	20/03/2019 16:33:39	JULI THOMAZ DE SOUZA	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Emenda1.pdf	08/03/2019 12:36:38	JULI THOMAZ DE SOUZA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	08/03/2019 11:20:14	JULI THOMAZ DE SOUZA	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	biorrepositorioJuli.pdf	11/11/2017 13:05:49	JULI THOMAZ DE SOUZA	Aceito
Outros	TermoDeAnuencialInstitucional.pdf	06/11/2017 09:11:58	JULI THOMAZ DE SOUZA	Aceito
Folha de Rosto	FolhaDeRosto.Pdf	06/11/2017 09:10:16	JULI THOMAZ DE SOUZA	Aceito
Outros	AnuenciaHcfmb.pdf	06/11/2017 09:00:50	JULI THOMAZ DE SOUZA	Aceito
Cronograma	CronogramaProjeto.pdf	06/11/2017 08:59:12	JULI THOMAZ DE SOUZA	Aceito
Declaração de Instituição e Infraestrutura	AnaliseDgaaHcfmb.pdf	06/11/2017 08:51:10	JULI THOMAZ DE SOUZA	Aceito
Orçamento	PlanilhaOrcamentariaUpeclin.Pdf	06/11/2017 08:50:05	JULI THOMAZ DE SOUZA	Aceito
Declaração de Instituição e Infraestrutura	AceiteUnipex.Pdf	06/11/2017 08:49:37	JULI THOMAZ DE SOUZA	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Endereço: Chácara Butignolli, s/n
 Bairro: Rubião Junior CEP: 18.618-970
 UF: SP Município: BOTUCATU
 Telefone: (14)3880-1609 E-mail: cep@fmb.unesp.br



Continuação do Parecer: 3.246.038

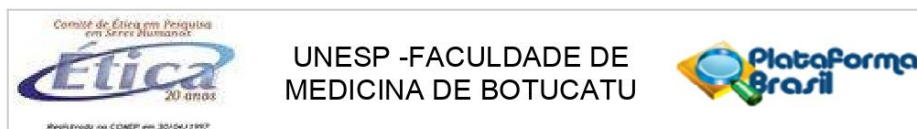
Não

BOTUCATU, 05 de Abril de 2019

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador(a))

Endereço: Chácara Butignolli, s/n	
Bairro: Rubião Junior	CEP: 18.618-970
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Telefone: (14)3880-1609	E-mail: cep@fmb.unesp.br

**ANNEX C - Approval document of Research Ethics Committee from Botucatu Medical School
- Amendment 2**



PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: INFLUÊNCIA DA SUPLEMENTAÇÃO DE PROTEÍNA E CREATINA NA CAPACIDADE FUNCIONAL, QUALIDADE DE VIDA E FUNÇÃO MUSCULAR APÓS ACIDENTE VASCULAR CEREBRAL

Pesquisador: JULI THOMAZ DE SOUZA

Área Temática:

Versão: 4

CAAE: 79844317.3.0000.5411

Instituição Proponente: Departamento de Clínica Médica

Patrocinador Principal: CONS NAC DE DESENVOLVIMENTO CIENTIFICO E TECNOLÓGICO

DADOS DO PARECER

Número do Parecer: 5.456.441

Apresentação do Projeto:

Trata-se de emenda para alteração de tamanho de amostra para 30 participantes e inclusão da população mulheres com 60 anos ou mais.

Objetivo da Pesquisa:

Já avaliados anteriormente e inalterados.

Avaliação dos Riscos e Benefícios:

Já avaliados anteriormente e inalterados.

Comentários e Considerações sobre a Pesquisa:

Trata-se de emenda para alteração de tamanho de amostra para 30 participantes e inclusão da população mulheres com 60 anos ou mais.

Considerações sobre os Termos de apresentação obrigatória:

Já avaliados anteriormente.

Nessa emenda foram apresentados: nova folha de rosto assinada e nova anuência da instituição.

Recomendações:

-

Conclusões ou Pendências e Lista de Inadequações:

Após análise em REUNIÃO ORDINÁRIA, o Colegiado deliberou APROVAÇÃO da EMENDA

Endereço: Chácara Butignolli, s/n

Bairro: Rubião Junior

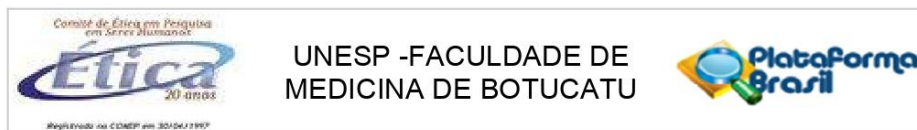
UF: SP

Município: BOTUCATU

Telefone: (14)3880-1609

CEP: 18.618-970

E-mail: cep@fmb.unesp.br



Continuação do Parecer: 5.456.441

apresentada.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado, em REUNIÃO ORDINÁRIA do Comitê de Ética em Pesquisa FMB/UNESP, realizada em 06/06/2022, a EMENDA apresentada encontra-se APROVADA.

Ao final da execução da pesquisa, o Pesquisador deverá enviar o Relatório Final de Atividades, na forma de Notificação, via Plataforma Brasil.

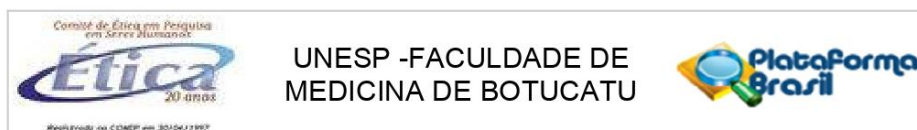
Atenciosamente,

Comitê de Ética em Pesquisa FMB/UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1921380_E2.pdf	26/04/2022 19:26:15		Aceito
Folha de Rosto	FolhaDeRostoEmenda3.pdf	26/04/2022 19:21:11	JULI THOMAZ DE SOUZA	Aceito
Declaração do Patrocinador	Patrocinador_CNPQ.pdf	26/04/2022 19:18:08	JULI THOMAZ DE SOUZA	Aceito
Outros	Anuencia_ambulatorioassinada.pdf	26/04/2022 19:15:13	JULI THOMAZ DE SOUZA	Aceito
Outros	Anuencia_unidadeassinada.pdf	26/04/2022 19:14:50	JULI THOMAZ DE SOUZA	Aceito
Outros	AnuenciaHcfmb_Emenda3.pdf	26/04/2022 19:14:03	JULI THOMAZ DE SOUZA	Aceito
Outros	Alteracoes_Emenda3.pdf	26/04/2022 19:12:35	JULI THOMAZ DE SOUZA	Aceito
Cronograma	Cronograma_emenda3.pdf	26/04/2022 19:10:48	JULI THOMAZ DE SOUZA	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_emenda3.pdf	26/04/2022 19:09:27	JULI THOMAZ DE SOUZA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	08/03/2019 11:20:14	JULI THOMAZ DE SOUZA	Aceito
Declaração de Manuseio Material Biológico / Biorepositório /	biorrepositorioJuli.pdf	11/11/2017 13:05:49	JULI THOMAZ DE SOUZA	Aceito

Endereço: Chácara Butignolli, s/n
 Bairro: Rubião Junior CEP: 18.618-970
 UF: SP Município: BOTUCATU
 Telefone: (14)3880-1609 E-mail: cep@fmb.unesp.br



Continuação do Parecer: 5.456.441

Biobanco	biorrepositorioJuli.pdf	11/11/2017 13:05:49	JULI THOMAZ DE SOUZA	Aceito
Declaração de Instituição e Infraestrutura	AnaliseDgaaHcfmb.pdf	06/11/2017 08:51:10	JULI THOMAZ DE SOUZA	Aceito
Orçamento	PlanilhaOrcamentariaUpeclin.Pdf	06/11/2017 08:50:05	JULI THOMAZ DE SOUZA	Aceito
Declaração de Instituição e Infraestrutura	AceiteUnipex.Pdf	06/11/2017 08:49:37	JULI THOMAZ DE SOUZA	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

BOTUCATU, 08 de Junho de 2022

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador(a))

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UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1609 **CEP:** 18.618-970
E-mail: cep@fmb.unesp.br