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REVIEW ARTICLE

^{31}P-MRS-Measured Phosphocreatine Recovery Kinetics in Human Muscles in Health and Disease—A Systematic Review and Meta-Analysis

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ABSTRACT

The noninvasive, in vivo measurement of postexercise phosphocreatine (PCr) recovery kinetics using 31-phosphorus magnetic resonance spectroscopy (^{31}P-MRS) is a highly prevalent method for assessing skeletal muscle energetics. However, ^{31}P-MRS methodology is notoriously laboratory-specific, leading to uncertainty about the normal range of PCr recovery kinetics among healthy individuals, as well as relationships with disease and demographic factors. This systematic review and meta-analysis characterized the normal range of PCr recovery kinetics from ^{31}P-MRS in human skeletal muscles across the lifespan, differences between healthy and those with muscle-related diseases, and relationships between intermuscular PCr recovery measurements and demographic factors. PubMed, Web of Science, Cochrane, and Google Scholar databases were searched for PCr recovery studies, which resulted in a final set of 128 studies eligible for meta-analysis. Studies were categorized into three muscle groups (forearm, upper leg, and lower leg) and further subdivided into three groups: diseased, control (the comparator group in studies of disease), and healthy (those recruited into studies that lacked a disease group). Only English-language studies were included. All statistical analysis was performed using Stata 17 software. Forest plots showed significant heterogeneity across PCr recovery time estimates and outlier study removal significantly reduced this heterogeneity. Greater age was associated with longer PCr recovery in upper leg muscles among both healthy ($\rho = 0.387$, $p < 0.05$) and diseased ($\rho = 0.733$, $p < 0.05$) individuals. Additionally, longer PCr recovery time was correlated with more acidic end-of-exercise pH in all three muscle groups among healthy individuals. In conclusion, skeletal muscle energetics as indexed by ^{31}P-MRS-based PCr recovery time is similar across three different skeletal muscle groups among healthy people. Common diseases significantly prolong PCr recovery times. Methodological heterogeneity has a significant impact on PCr recovery time measurements in this literature. Greater age and more acidic pH increase PCr recovery time among healthy people.

Abbreviations: ^{31}P-MRS, ^{31}phosphorus-magnetic resonance spectroscopy; ADP, adenosine diphosphate; ATP, adenosine triphosphate; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; CSI, chemical shift imaging; DRESS, depth-resolved surface coil spectroscopy; DS, Down syndrome; FC, Fontan circulation; FLORET, Fermat looped, orthogonally encoded trajectories; HSCT, hematopoietic stem cell transplant; HT, hyperthyroid; ISIS, image-selected in vivo spectroscopy; JD, juvenile dermatomyositis; LBW, low birth weight; MCF, mild cystic fibrosis; MH, malignant hyperthermia; MM, mitochondrial myopathy; MRSI, magnetic resonance spectroscopic imaging; NA, not available; NAFLD, nonalcoholic fatty liver disease; NOE, nuclear Overhauser enhancement; PAD, peripheral artery disease; PCr, phosphocreatine; Pi, inorganic phosphate; RF, radio frequency; SCA, sickle cell anemia; SCI, spinal cord injury; semi-LASER, semi–localization through adiabatic selective refocusing; STEAM, stimulated echo acquisition mode.

1 | Introduction

The human muscle system comprises three primary compartments (cardiac, smooth, and skeletal), with skeletal muscles being crucial for our ability to move and maintain posture and balance [1]. Different skeletal muscles differ in their structure, composition, and perfusion [2]. As one of the most energy-consuming organs, the skeletal muscle significantly contributes to the maintenance of energy homeostasis in the body [3]. Its remarkable adaptability to changes in contractile activity [4, 5] enables the skeletal muscle to support sustained physical exertion, which in turn is a key determinant of physical functioning. In addition, several diseases (including musculoskeletal conditions as well as diabetes, obesity, and cancer) feature disruptions in skeletal muscle energy substrate processing, for reasons that are currently under active investigation [2, 6]. Because of its importance in health and disease, techniques that evaluate aspects of skeletal muscle energetics have become vital research tools.

We use the term "muscle metabolism" to refer specifically to adenosine triphosphate (ATP) production in muscle that is required to fulfill current energy demands. The skeletal muscle obtains energy by oxidizing metabolic fuel substrates including carbohydrates, lipids, and proteins. In the mitochondria [7], these oxidative processes yield energy in the form of ATP, a molecule with a high-energy phosphoanhydride bond. ATP serves as a substrate for muscle contraction, being hydrolyzed into adenosine diphosphate (ADP) and inorganic phosphate (Pi) by the enzyme ATPase, releasing energy in the process [8, 9]. This energy is utilized through phosphorylation, a process where a free Pi is transferred to another molecule, essential for all muscular work. For example, ATP powers muscle contraction by transferring a phosphate group to contractile proteins, enabling muscle fiber contraction. During muscle activity, the initial ATP stores are rapidly depleted, necessitating ATP resynthesis. This process involves the recombination of ADP and Pi to regenerate ATP, often accelerated by the rapid creatine kinase reaction between phosphocreatine (PCr) and ADP. Muscle cells efficiently store energy as PCr, a less reactive molecule that acts as a reserve due to the rapid and reversible nature of the creatine kinase reaction. As ATP is hydrolyzed to ADP and Pi during muscle contraction, the creatine kinase reaction quickly restores ATP by transferring a phosphate group from PCr to ADP. This mechanism operates much faster than other metabolic pathways, ensuring an immediate supply of ATP to sustain muscle activity. Following the cessation of exercise at an intensity lower than that of the anaerobic threshold, ATP production surpasses its consumption, triggering PCr resynthesis via an aerobic process of mitochondrial oxidative phosphorylation. The relationship between PCr resynthesis and ATP production in muscle cells makes it possible to use PCr dynamics—specifically, the rate constant of PCr recovery—as a proxy measure of skeletal muscle mitochondrial oxidative phosphorylation capacity [10–12]. Faster PCr recovery is considered favorable for muscle performance in practical settings, as it suggests swifter replenishment of the PCr pool, facilitating readiness for conversion to ATP to meet subsequent exercise demands. In contrast, slower recovery suggests a prolonged duration to achieve a comparable PCr pool size and thus a propensity toward depletion.

The leading method for measuring PCr, Pi, and ATP concentrations in vivo, noninvasively, is phosphorus magnetic resonance spectroscopy (^{31}P-MRS). The chemical environment of the ^{31}P nuclei in these species of molecules causes each species to resonate at its own specific frequency when excited by radio frequency (RF) energy. For this reason, each molecular species occupies a specific frequency in the spectrum measured by ^{31}P-MRS. The amount of signal in the spectrum at that specific frequency is an indicator of the relative abundance of that molecular species in the tissue under study, and absolute abundance can be calculated through corresponding measurements of a well-characterized molecular phantom. Resting-state ^{31}P-MRS exams measure the abundances of molecular species of interest in a static fashion to characterize the basal state of muscle tissue. Dynamic ^{31}P-MRS exams allow measurement of how these abundances evolve over time. The method can be used to assess the rate of PCr replenishment after the imposition of exercise or other metabolic stimuli.

Besides the time constant of PCr recovery [13–17], muscle mitochondrial oxidative capacity can also be summarized in terms of Pi/PCr ratios [13, 14, 18]. Although both readouts can provide useful information, PCr recovery rate offers the advantage that it does not require precise measurements of work intensity or muscle mass. Instead, it only requires sufficient PCr depletion (provided the pH does not decrease significantly [19, 20]) through a brief (e.g., 20–30 s), intense exercise bout and good time resolution (e.g., one spectrum every 5 s) [21, 22]. Because of this relative ease of measurement, PCr recovery kinetics are more common in the literature than Pi/PCr ratios.

Measuring PCr recovery kinetics is not without challenges, however. Methodology varies widely from study to study, and there are no widely accepted normal ranges of measurement values, consensus about measurement confounders (such as age), or consensus about disease effects. PCr recovery rate is confounded by muscle pH, which decreases over the course of an extended contractile bout [23, 24], but neither the exact relationship between pH and PCr recovery rate nor conventions for how to handle pH variations analytically are well understood. Other methodological differences include different pulse sequences, exercise protocols, and MR scanner magnetic field strengths. The lack of consensus on these aspects of PCr methodology has limited the study of skeletal muscle metabolism by making it especially difficult for laboratories to determine which ^{31}P-MRS methodological choices to make or how to position their findings within the broader context of the muscle physiology field. Therefore, this systematic review and meta-analysis aims to determine whether there is consensus on normal ranges for PCr recovery kinetics in three different skeletal muscle groups, as well as whether there are consensus relationships between these measurements (intermuscular differences) and age, end-of-exercise pH, and diseases. However, investigating intramuscular differences in PCr recovery within each skeletal muscle group is beyond the scope of this review.

2 | Methods

2.1 | Study Strategy

The search encompassed Google Scholar, PubMed, Web of Science, and Cochrane databases for studies published between

January 1990 and February 2023. Only English search terms for different muscle groups (pcr recovery kinetics AND forearm muscle exercise AND 31P MRS protocols, pcr recovery kinetics AND quadricep muscle exercise AND 31P MRS protocols, and pcr recovery kinetics AND plantar flexor muscle exercise AND 31P MRS protocols) were used, and only English language studies were included.

2.2 | Search Selection

Inclusion of a study required an exercise challenge paradigm targeting skeletal muscle PCr recovery kinetics using ^{31}P-MRS within healthy or diseased children or adults across the entire lifespan. Eligible studies could be experimental, diagnostic, correlational, descriptive, observational cohort, and case–control human studies, regardless of whether randomization was used. No restrictions were applied regarding intervention protocol, duration, or setting. Studies were excluded if they did not involve humans, did not use ^{31}P-MRS to measure PCr kinetics, only measured skeletal muscle PCr kinetics during exercise, or measured steady-state PCr metabolites without exercise.

The lead researcher (M.S.) and researcher (A.J.) reviewed the articles independently and categorized all identified studies as relevant, irrelevant, duplicates, or ambiguous. The studies in the ambiguous group were then discussed with a third researcher (O.C.) and the relevance was resolved through discussion. The abstracts and full text of the remaining studies were reviewed by the lead researcher (M.S.) and cross-checked by the second researcher (A.J.), resulting in a final set of relevant studies.

2.3 | Data Categorization and Extraction

The lead researcher (M.S.) and researcher (A.J.) reviewed the included studies and categorized them by muscle group. Studies for each muscle group were subsequently categorized into three groups—healthy, control, and disease—based on participants' physiological health. The control group consists of individuals who were nominally free of diseases and were recruited for the purpose of comparison to a disease group in a comparative study. The healthy group consists of individuals who were nominally free of disease and were recruited into a study focusing on skeletal muscle physiology among healthy individuals. After completing the study categorization, data abstraction was performed by both researchers (M.S. and A.J.), and the results were compared for any discrepancies through discussion and recommendations.

2.4 | Inclusion of Studies With Differing Diseases

The disease groups from studies of differing diseases were grouped together in the statistical analysis because the effects of their various disease processes converged onto a shared biological pathway of mitochondrial dysfunction, which is what PCr recovery rate measurements capture. Additionally, the number of studies available for any individual disease was too small to allow for separate analyses of each disease, thus necessitating our combined approach to ensure statistical validity and meaningful conclusions.

2.5 | Meta-Analysis

The three goals of the meta-analysis were to evaluate pooled means and standard deviations in PCr recovery rate within the three separate muscle groups among healthy individuals, to assess pooled differences in mean PCr recovery rate between control and diseased groups, and to assess consensus correlations between PCr recovery rate and demographic and physiological factors such as age and pH. Random effect analysis was used throughout to weigh each study and to control potential heterogeneity [25]. The mean difference statistic was used for effect size calculations. Single-group mean analysis was performed for studies exclusively involving healthy participants, and two-group mean analysis was used for studies comparing diseased and control participants. Interstudy heterogeneity was assessed using the Cochran Q statistic, and the extent of this heterogeneity was quantified using the I^2 statistic [26]. This measures the percentage of variation in effect size attributed to heterogeneity. I^2 values of 0.25, 0.50, and 0.75 indicate low, moderate, and high levels of heterogeneity, respectively. A significance threshold of $p < 0.05$ was employed to determine if the studies were heterogeneous. In cases where the Q-statistic showed significance, a Galbraith plot was utilized to identify studies "called outliers" responsible for the most significant heterogeneity. Specific criteria used to identify an outlier include when a study's 95% confidence interval lies outside the 95% confidence interval band of their pooled effect. We also explored publication bias, whereby studies are less likely to be published when results are nonsignificant or characterized by small effects. It was examined using Egger's test and a funnel plot. This approach enabled us to investigate the potential underlying causes for the heterogeneity observed [27]. We further tested the robustness of the findings from the meta-analysis via a sensitivity analysis that excluded studies utilizing nonstandard methodologies, including multiple sequential exercise bouts per individual and less-common MRS pulse sequences (STEAM, depth-resolved surface coil spectroscopy [DRESS], image-selected in vivo spectroscopy [ISIS], semi–localization through adiabatic selective refocusing [semi-LASER], Fermat looped, orthogonally encoded trajectories [FLORET], magnetic resonance spectroscopic imaging [MRSI], and 2D-CSI). In cases where the data remained heterogeneous even after exclusion, we employed meta-regression using a random effect model. This helped predict the effect size based on the study sample size and weights, providing a more nuanced understanding of the results.

Correlation analysis was performed to test our secondary hypotheses that greater age is associated with a slower PCr recovery rate and that more acidic end-of-exercise pH is associated with a slower PCr recovery rate, across all three muscle groups. The strength of relationships between variables was assessed by study sample weighted Spearman correlation. A correlation coefficient $r_S = 0.35–0.49$ was interpreted empirically as low, 0.5–0.79 as moderate, and 0.8 or greater as high.

The statistical analysis was conducted using Stata Statistical Software, Release 17 (StataCorp, College Station, TX). To

determine the methodological sources of heterogeneity in PCr recovery rate measurements, we applied the analysis of variance (ANOVA) method. One-way ANOVA was used to assess whether specific methodological choices, such as exercise prescription, or parameters like the coil size (diameter in centimeters), the number of sampling points, temporal resolution, and recovery periods were associated with differences in PCr recovery rates. Additionally, a two-way ANOVA was conducted to evaluate whether interactions between these factors had a significant effect on PCr recovery rates. Post hoc analyses were performed

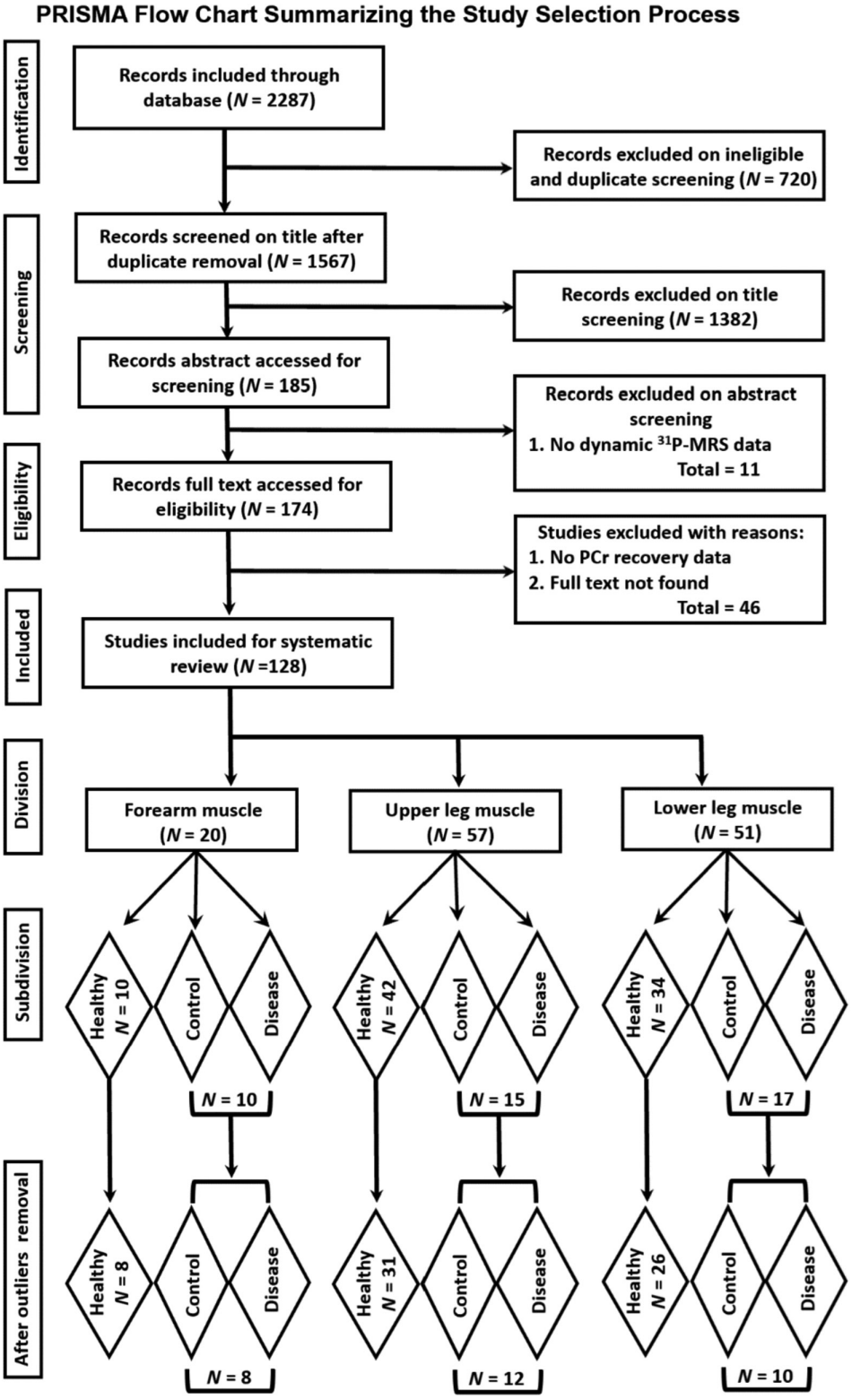

FIGURE 1 | PRISMA flowchart showing the study selection process. Ineligible: These are the studies that have the characteristics that disqualify them from inclusion in a review. Duplicate: These are the studies that share the same author, title, publication date, volume, issue, and start page information. Title/abstract: These are the studies whose title/abstract does not have characteristics of the inclusion criteria.

using pairwise *t*-tests with Bonferroni correction for multiple comparisons.

3 | Results

3.1 | Literature Search

The search was conducted and completed in March 2023. A PRISMA flowchart [156] of the study selection process is provided in Figure 1. In total, 2287 relevant studies were initially identified, screening for duplicates reduced this set to 1567, screening for off-topic titles further reduced this set to 185, abstract screening reduced this set to 174, and full-text screening reduced the set to the final set of 128 analyzed articles [28–117, 121–154, 157–160].

3.2 | Overall Summary of Participant Characteristics

Detailed information about each included study is provided in Tables S1–S6. Participants' age covered almost the entire lifespan from childhood to old age [120]. The studies collectively include individuals from both sexes, a wide range of training and health status, and a broad range of exercise performance. Most studies were performed in northern European countries and the United States (Figures 2 and 3).

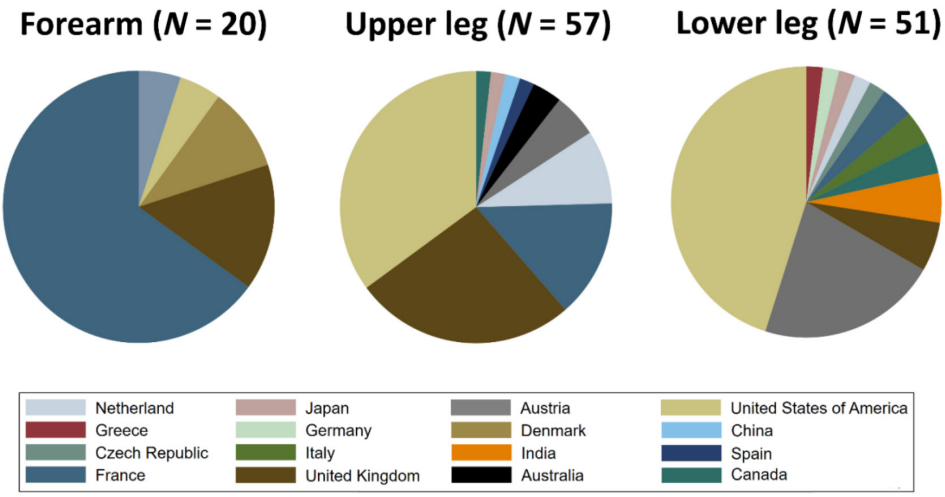

FIGURE 2 | Pie charts depicting the global distribution of the included ^{31}P-MRS studies [28–155] of various muscle groups.

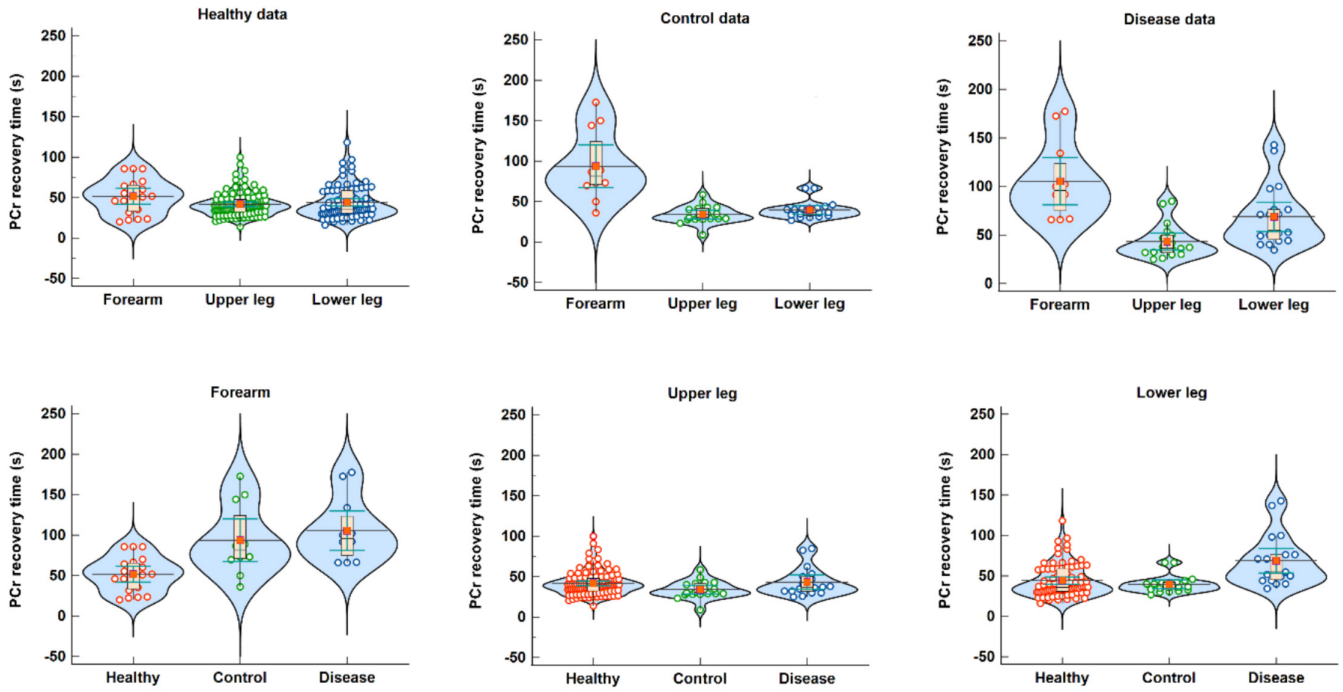

FIGURE 3 | Violin plots showing density estimates together with box plots of the mean PCr recovery rate values of included studies across different muscle groups. The black horizontal line in the box plot indicates the mean value with error bars (in dark green).

3.3 | Overall Summary of MR Scanner and Sequence Characteristics

All included studies used 1.5- to 7-T MRI field strength and RF volume [107, 127, 133, 139] or surface coils of varying diameters (ranging from 2.5–27 cm) from various manufacturers. Most studies used pulse-and-acquire sequences without spin echo for ^{31}P-MRS measurements [28–101, 103, 105, 108, 111, 113, 114, 116, 121, 123, 125, 126, 128, 131, 134, 141, 143–154, 157–160]. A few other studies employed methods such as STEAM [115], DRESS [110, 122], ISIS [142], semi-LASER [102, 106, 124, 127, 132, 133], FLORET [107, 112], MRSI, and 2D-CSI [104, 109]. Sequence parameters including repetition time (0.5–8 s), the number of averages (1–32 averages), temporal resolution (2–60 s), exercise time (24–600 s), and recovery time (3–20 min) varied significantly across included studies. Nuclear Overhauser enhancement (NOE) can improve signal-to-noise ratio (SNR) and reproducibility, and several studies [90, 96, 103, 106, 126, 128, 140, 147] have utilized NOE to achieve these improvements. A one-way ANOVA revealed no significant effect of NOE on PCr recovery rates (ANOVA; $p = 0.25$). However, because of the limited number of studies that included NOE, the ANOVA results may be biased. Further research is needed to validate these findings.

3.4 | Overall Summary of Muscle Characteristics and Exercise Methods

Most ^{31}P-MRS studies prioritized large superficial flexor muscles to maximize signal recovery from the surface coils utilized. Specifically, the flexor digitorum superficialis was often studied in the forearm, the rectus femoris and vastus lateralis were preferred in the upper leg, and the gastrocnemius and soleus muscles were studied in the lower leg. Preferred exercise methods for forearm flexion included finger and wrist flexion, leg flexion, and extension were utilized for the upper leg, and plantar flexion and extension were used for the lower leg. The experimental protocol was prescribed in a dynamic rest–exercise–recovery fashion, and the duration of these phases may vary depending on the goal of the study.

3.5 | Forearm Studies

Characteristics of the 20 forearm studies [28–43, 157–160] are provided in Tables S1 and S2. Most studies were small (median sample size = 13). Overall, 70.8% of the participants were male (range 0%–100%) with 11 studies recruiting only males [28–33, 36, 39, 40, 43, 158, 160], eight studies recruiting both males and females [30, 35, 37, 38, 41, 42, 157, 159], and only one female-only study [34]. The mean participant age was 37.05 years, with 18 studies targeting only adults [28–43, 157, 159], two studies enrolling both children and adults [158, 160], and two studies enrolling only older adults [39, 40]. Nineteen studies included untrained individuals, and only one study [28] included resistance-trained participants. All studies utilized dynamic rest–exercise–recovery protocols. All case–control studies compared controls and patients with muscular myopathies (including myalgia [34, 37], malignant hyperthermia [36, 41], and chronic renal failure [39, 40]). The exercise stimulus in all but four studies in Tables S1 and S2 was finger flexion; the remainder [28, 34–36] utilized wrist flexion. Fourteen studies prescribed a constant flexion workload [30, 32–35, 37–40, 43–45, 157, 158, 160], whereas six involved incremental workloads [28, 29, 31, 34, 39, 40]. One study prescribed exercise until volitional fatigue [28]; all others prescribed a fixed time interval of exercise.

3.5.1 | Primary Outcomes

The simple mean (standard deviation) of the reported PCr recovery rates from all studies for healthy, control, and diseased groups were 51.69 (21), 93.74 (42), and 105.42 (38) s (Figure 3), respectively. The simple mean end-of-exercise pH in the healthy, control, and diseased groups were 6.65 (0.17), 6.51 (0.11), and 6.5 (0.16).

In the meta-analysis, the sample size weighted mean PCr recovery rate among healthy individuals was 38.74 (21) s, with an I^2 value of 41.4%, $\text{tau}^2 = 140.4$, and $p = 0.047$, indicating low magnitude but significant ($p < 0.05$) heterogeneity (Table 1). The asymmetrical funnel plot (Figure S2A) and the results of Egger's test (intercept = 1.23, $p = 0.002$) suggested potential

TABLE 1 | Heterogeneity statistics and pooled mean PCr recovery rate estimate for the single group mean meta-analysis among healthy individuals, before and after outlier (study outside 95% CI in Galbraith plot) removal.

Test for residual heterogeneity (healthy data)					
Before outlier studies removal	**I^2 (Q statistic)**	**Tau^2**	**Degrees of freedom**	**Statistical significance**	**Pooled mean PCr recovery rate (s)[a]**
Forearm	41.4	140.41	19	0.0472	38.74 (21)
Upper leg	52.2	46.97	107	< 0.0001	37.23 (12)
Lower leg	47.8	83.28	81	< 0.0001	37.73 (15)
After outlier studies removal					
Forearm	0.0	0.0	16	0.8425	34.41 (17)
Upper leg	2.66	2.3	71	0.99	35.66 (9)
Lower leg	0.0	0.0	52	0.74	33.75 (10)

[a]The values are mean (SD) in seconds.

TABLE 2 | Heterogeneity statistics and the pooled group mean differences between control and disease groups for comparative studies, before and after outlier removal.

Test for residual heterogeneity (control vs. disease data)					
Before outlier studies removal	**I^2 (Q statistic)**	**Tau^2**	**Degrees of freedom**	**Statistical significance**	**Control vs. disease mean difference (s)[a]**
Forearm	49.95	266.05	11	0.0076	15.96 (22)
Upper leg	41.03	12.41	17	0.002	6.63 (6)
Lower leg	83.84	205.89	18	<0.0001	22.09 (16)
After outlier studies removal					
Forearm	1.37	4.59	9	0.5815	25.18 (15)
Upper leg	0.0	0.0	13	0.6138	5.38 (4)
Lower leg	45.08	27.18	10	0.04	16.74 (7)

[a]The values are mean (SD) in seconds.

publication bias. Additionally, the Galbraith plot (Figure S2A) identified two specific studies [31, 33] as significant contributors to this bias. Sensitivity analyses excluding these two studies resulted in a similar pooled mean PCr recovery rate of 34.41 (17) s, greater statistical significance, and nonsignificant heterogeneity (Figure S1B, $I^2 = 0.0\%$, tau$^2 = 0.0$, and $p = 0.843$).

The pooled mean difference between the control and diseased groups was 15.96 (22) s, which was significant (95% confidence interval: 1.70–30.22, $p = 0.03$). The I^2 value was 49.95%, indicating moderate magnitude and statistically significant ($p < 0.05$) heterogeneity (see Table 2). The asymmetrical funnel plot (Figure S2B), along with the results of Egger's test (intercept = −0.62, $p = 0.5$), indicated a potential presence of publication bias. Furthermore, the Galbraith plot (Figure S2) identified two specific studies [42, 43] as significant contributors to this bias. Sensitivity analyses excluding these two studies had a greater pooled mean difference, greater statistical significance, and lower heterogeneity (Figure 4, top, pooled MD = 25.18 (15) s, $I^2 = 1.37\%$, tau$^2 = 4.59$, and $p = 0.582$).

3.5.2 | Secondary Outcomes

Among the studies of healthy participants, age and PCr recovery rate were not significantly correlated ($p = 0.54$). Lower end-of-exercise pH was strongly and significantly correlated with greater PCr recovery rate ($r = -0.726$, $p < 0.05$).

In both the control and diseased groups of case–control studies, age, and PCr recovery rate were not significantly correlated ($p = 0.1152$ and $p = 1.0$). Neither were end-of-exercise pH and PCr recovery rate correlated in either control or diseased groups ($p = 0.90$ and $p = 0.348$).

3.6 | Thigh/Upper Leg Studies

Tables S3 and S4 provide a description of individual characteristics within the 57 included studies. The median sample size across studies was 9, encompassing a total of 1895 participants. Overall, 66.1% of participants were male (range 0%–100%), with 13 studies exclusively recruiting males [48, 53, 54, 59, 60, 64, 67–69, 73, 76, 85, 92], and the remaining studies including both males and females. Eleven studies [52, 57, 58, 66, 74, 78, 79, 81–83] reported gender and/or age group-specific results. The mean participant age was 34.6 years, with 46 studies exclusively targeting adults, five focusing solely on children [65, 91, 92, 95, 100], three including both children and adults [52, 74, 79], two involving both young and older adults [82, 83], and six [57, 63, 70, 72, 84, 86] exclusively involving older participants. One study [75] had a diagnostic nature, five [44, 54, 55, 69, 82] were correlational, 13 [45, 46, 49, 51, 53, 59, 61–63, 66, 76, 84, 85] were experimental, and 21 [47, 48, 50, 52, 56, 57, 60, 65, 67–74, 78–81, 83] were descriptive, whereas 15 were case–control studies [86–100]. All studies utilized isometric or isokinetic leg/knee extension exercises of the quadriceps muscle in a rest–exercise–recovery protocol. Nine out of 57 studies included muscle flexion exercises at incremental workload [47, 48, 52, 68, 91–93, 95, 97], whereas the rest used a constant workload. In 10 studies, the exercise continued until volitional fatigue [46, 48, 52, 62, 63, 73, 74, 91, 92, 95], whereas in all other studies, exercise was performed for a fixed time interval. One included study [59] used the DRESS method to measure PCr kinetics data instead of a simple pulse acquire sequence.

3.6.1 | Primary Outcome

The simple mean (standard deviation) of the reported PCr recovery rates from all studies for healthy, control, and disease groups was 41.9 (15), 33.8 (11), and 43.2 (18) s, respectively. The simple mean end-of-exercise pH for healthy group participants was 6.91 (0.16), whereas for the control and disease groups, these values were 6.86 (0.16) and 6.85 (0.17), respectively.

Meta-analysis revealed a sample size weighted mean PCr recovery rate of 37.23 (12) s with an I^2 value of 52.24%, a tau^2 of 46.97, and a $p < 0.001$, indicating a statistically significant ($p < 0.05$) moderate level of heterogeneity in the data (see Table 1 and Figure S1E). The funnel plot (Figure S2C) and the results of

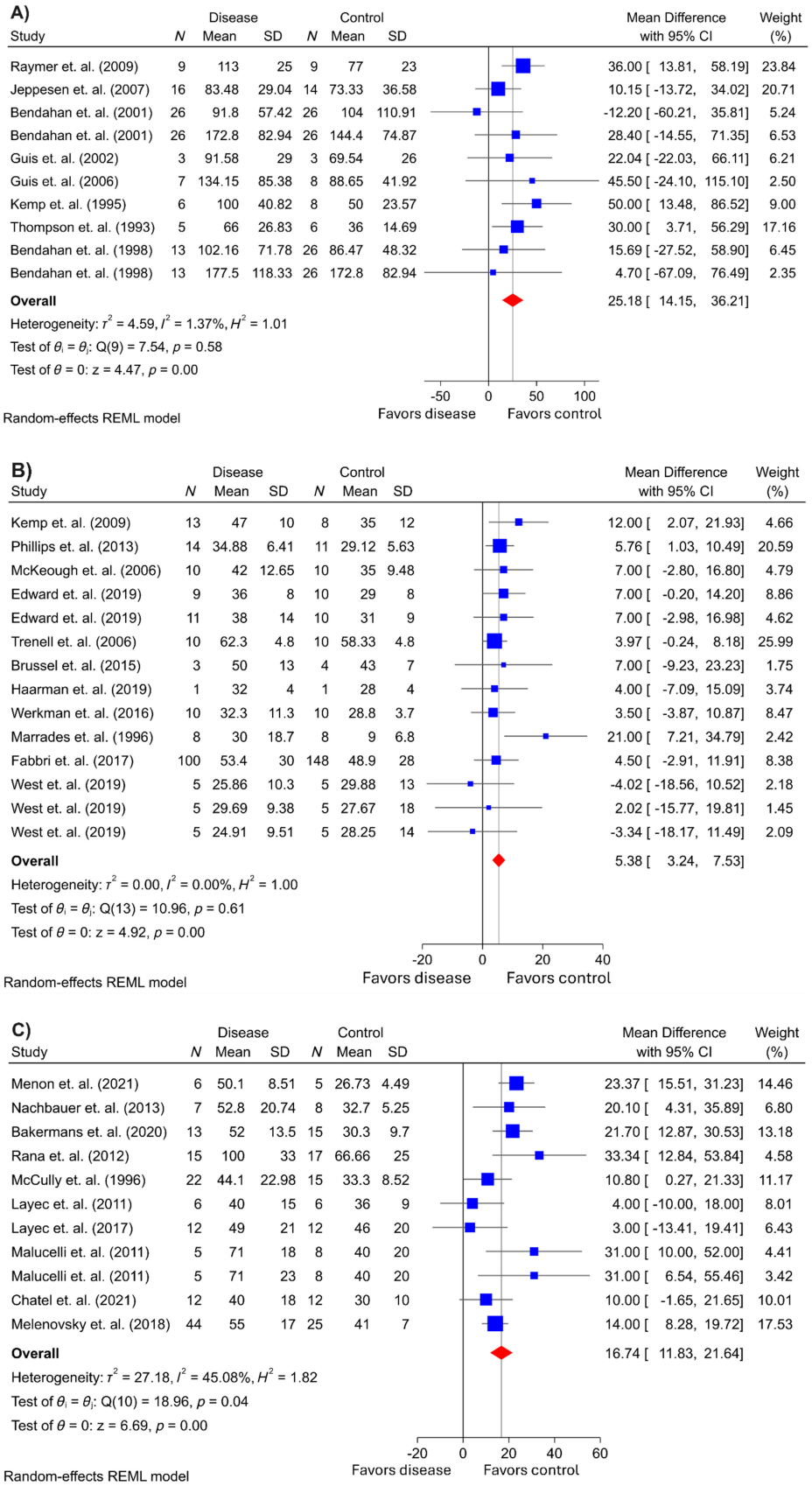

FIGURE 4 | Random effect model—forest plots (after outlier studies removal) for comparative studies for (A) forearm, (B) upper leg, and (C) lower leg muscle groups. The mean difference between disease and control studies is used as a measure of effect size, which is represented as the blue squares along with their respective confidence intervals. The size of the blue square representing effect size is proportional to the study weight. The red diamond at the bottom represents the overall mean difference or effect size across these studies. Cochran *Q* statistics were also reported at the bottom of each plot along with significance for heterogeneity analysis.

Egger's test (intercept = 1.46, $p = 0.001$) further suggest potential publication bias. Additionally, the Galbraith plot (Figure S2C) identified 11 studies [53, 58, 59, 64, 68, 70, 73, 75, 76, 80, 83] as significant contributors to this bias. Sensitivity analyses excluding these 11 studies resulted in a similar pooled mean PCr recovery rate of 35.66 (9)s, greater statistical significance, and very low heterogeneity (Figure S1F, $I^2 = 2.66\%$, $\text{tau}^2 = 2.3$, and $p = 0.99$).

Two-group meta-analysis revealed the sample size weighted mean PCr recovery rate difference between the control and disease groups as 6.63 (6)s, which was statistically significant (95% confidence interval: 3.85–9.41, $p < 0.05$). The I^2 value was 41.03%, indicating a moderately significant ($p < 0.05$) heterogeneity (see Table 2 and Figure S1G). The asymmetrical funnel plot (Figure S2D), along with the results of Egger's test (intercept = 1.32, $p = 0.056$), indicated a potential publication bias. Furthermore, the Galbraith plot in Figure S2D identified four specific studies [93, 94, 96, 98] as confounding factors contributing to this bias. Sensitivity analyses excluding these four studies resulted in a lower pooled mean difference of 5.38 (4)s, greater statistical significance with very low heterogeneity (Figure 4, middle, $I^2 = 0.0\%$, $\text{tau}^2 = 0.0$, and $p = 0.61$).

3.6.2 | Secondary Outcomes

In the studies of healthy individuals, a significant correlation was found between greater mean PCr recovery rate and greater mean age ($p < 0.05$). Furthermore, a significant correlation ($p < 0.05$) was also observed between greater mean PCr recovery rate and more acidic mean end-of-exercise pH.

In comparative studies, within the control group, neither mean age ($p = 0.052$) nor end-of-exercise pH ($p = 0.99$) was significantly correlated with mean PCr recovery rate. Within the diseased group, a significant correlation was observed between greater mean PCr recovery rate and greater mean age ($p < 0.05$) of the participants. However, a nonsignificant correlation was observed between mean PCr recovery rate and mean end-of-exercise pH ($p > 0.357$).

3.7 | Lower Leg/Calf Muscle Studies

Tables S5 and S6 provide the study-by-study description of the 51 calf muscle studies. Most of the studies were small (median sample size was 12), with 1345 participants in total. Overall, 66.9% of the participants were male (range 0%–100%) with 16 studies recruiting only males [103, 107, 108, 110, 114, 116, 117, 125, 126, 128, 130, 131, 135, 148, 149, 151]; two, only females [137, 153]; and the remaining studies, both males and females. The mean age of participants was 33.6 years, in all studies except one [146] targeting only adult participants and five [116, 122–124, 141] targeting young and old adults. All except two studies included only untrained participants [126, 128], one included only physically active volunteers [123], and none of the studies included both trained and untrained participants. In total, six studies [138, 139, 141, 149–151] were observational, eight [104, 122, 126, 127, 132, 133, 136, 137] were correlational, 13 [101, 102, 107–111, 114, 115, 124, 135, 140, 142] were experimental, and 24 [103, 105, 106, 110, 113, 116, 117, 121, 123, 125, 128–131, 134, 143–148, 152–154] were descriptive in nature. All studies utilized isometric or isokinetic plantar flexion exercise of calf muscles (gastrocnemius or soleus) in a rest–exercise–recovery protocol. Four out of 51 studies included muscle flexion exercises at incremental workload [135, 140, 147, 148], and the rest at constant workload. In seven studies, the exercise was continued till volitional fatigue [115, 125, 136, 140, 141, 143, 148], whereas in all other studies, exercise was performed for a fixed time interval. Two included studies [110, 122] used DRESS, two [104, 109] used chemical shift spectroscopic imaging (CSI), five [106, 124, 127, 132, 133] used semi-LASER, two [107, 112] used FLORET, one [142] used ISIS, and one [115] used STEAM to measure PCr kinetics data as opposed to a simple pulse acquire sequence.

3.7.1 | Primary Outcomes

In the healthy, control, and diseased groups, the simple mean of reported PCr recovery rates from all studies were 44.3 (20), 39.8 (11), and 68.7 (31)s (Figure 3), with mean end-of-exercise pH values of 6.91 (0.16), 6.96 (0.09), and 6.96 (0.12), respectively.

In the meta-analysis, studies within the healthy group exhibited statistically significant moderate heterogeneity, with a sample size weighted mean PCr recovery rate of 37.73 (15)s, an I^2 value of 47.81%, a tau^2 of 83.28, and $p < 0.05$ (see Table 1 and Figure S1I). The funnel plot (Figure S2E) and the results of Egger's test (intercept = 1.23, $p = 0.001$) suggested potential publication bias. The Galbraith plot (Figure S2E) identified eight specific studies [102, 107, 109, 114, 117, 121, 132, 133] as significant contributors to this bias. Sensitivity analyses, excluding these eight studies, resulted in a lower pooled mean PCr recovery rate of 33.75 (10)s, greater statistical significance with very low heterogeneity (Figure S1J, $I^2 = 0.0\%$, $\text{tau}^2 = 0.0$, and $p = 0.74$). Additionally, a highly significant sample size weighted mean difference of 22.09 (16) (95% confidence interval: 14.42–29.76, $p < 0.05$) was observed between the control and diseased groups. An I^2 value of 83.84% indicated a high level of significant heterogeneity ($p < 0.05$). The skewed funnel plot (Figure S2F) and Egger's test results (intercept = 2.93, $p < 0.05$) suggested a potential presence of publication bias. The Galbraith plot in Figure S2F identified seven specific studies [138, 141, 142, 144, 146, 148] as contributors to this bias. Sensitivity analyses, excluding these seven studies (see Figure 4, bottom), still revealed a significant moderate level of heterogeneity in the findings, pooled mean difference = 16.74 (7), $I^2 = 45.08\%$, $\text{tau}^2 = 27.18$, and $p < 0.05$ (see Table 2 and Figure S1L). Subsequently, a meta-regression analysis utilizing the random effect model was also performed to assess the influence of study size and weight on the mean difference. The results, detailed in Table 3, indicated that under the null hypothesis, none of the covariates exhibited a significant impact (intercept ≈ 0, and $p > 0.05$) on the mean difference.

3.7.2 | Secondary Outcomes

Within healthy groups (Table S5), the relationship between mean PCr recovery rate and age was not statistically significant ($p = 1.0$). However, a significant correlation was observed

TABLE 3 | Random effect model—Meta-regression results for PCr recovery rate mean difference and study sizes and weights.

Random effects, *Z* distribution						
	Coefficient	**Standard error**	**95% lower**	**95% upper**	***z* value**	***p***
Intercept	19.42	5.18	9.26	29.58	3.74	0.00
Study size	−0.17	0.24	−0.63	0.30	−0.69	0.49
Study weight	49.63	128.11	−201.46	300.72	0.39	0.70

between greater mean PCr recovery rate and greater mean end-of-exercise pH ($p < 0.05$). For both control and diseased groups, there was a nonsignificant correlation ($p = 1.0$) between mean PCr recovery rate and age as well as mean PCr recovery rate and mean end-of-exercise pH ($p = 1.0$).

3.8 | PCr Recovery Comparison of Three Muscle Groups

A one-way ANOVA showed that PCr recovery times across the three muscle groups (forearm, upper leg, and lower leg) were not significantly different (ANOVA; $p = 0.07$) in healthy individuals but were significantly different in control (ANOVA; $p < 0.001$) and diseased individuals (ANOVA; $p < 0.001$). Additionally, post hoc tests revealed that the forearm muscle group had significantly slower PCr recovery times ($p < 0.001$) compared to both the upper and lower leg muscles in control and diseased groups. Furthermore, various disease processes causing mitochondrial dysfunction were found to significantly affect PCr recovery times across the forearm, upper leg, and lower leg muscle groups (ANOVA; $p = 0.046$). However, none of the muscle groups crossed the significance threshold ($p < 0.017$) during post hoc comparisons.

3.9 | Methodological Sources of Heterogeneity in the Data

Several methodological sources of heterogeneity in PCr recovery rate measurements were identified. These include exercise protocols, which influence muscle recruitment; coil size, diameter in centimeters, affecting whether data originate from superficial or deep muscles; and the number of sampling points, temporal resolution, and recovery period. These latter factors are critical for accurately mapping PCr recovery to a steady state and for avoiding data truncation or bias during curve fitting. A one-way ANOVA was conducted to examine the effect of these specific factors on PCr recovery times.

The exercise protocols utilized in the studies were categorized into three subgroups based on exercise intensity and duration: low-to-moderate (LTM) exercise intensity, moderate-to-high (MTH) exercise intensity, and incremental exercise till exhaustion (ETE). The PCr recovery times across these subgroups were found to be significantly different (ANOVA; $p < 0.004$). Post hoc pairwise comparisons were conducted using the Bonferroni correction to account for multiple comparisons. The test showed that PCr recovery times for LTM (mean = 37 s, SD = 6.6) and MTH (mean = 40.5 s, SD = 15) exercise intensity protocols were significantly faster (ANOVA; $p < 0.001$) compared to ETE (mean = 55.7 s, SD = 20), with an adjusted p value of 0.017. These findings suggest that both exercise intensity and duration influence PCr recovery times. Furthermore, a greater surface coil diameter was significantly (ANOVA; $p < 0.001$) correlated with slower PCr recovery times. In our analysis, sampling points ranged from 8 to 450, temporal resolution from 2 to 60 s, and recovery periods from 180 to 1200 s. A higher number of sampling points (ANOVA; $p < 0.001$), finer temporal resolution (ANOVA; $p < 0.001$), and longer recovery periods (ANOVA; $p < 0.001$) were significantly correlated with slower PCr recovery. However, no significant interaction effects were found among these methodological sources. Collectively, these methodological variations significantly impact the reliability and reproducibility of PCr recovery measurements.

4 | Discussion

In this review, we investigated a total of 128 ^{31}P-MRS studies measuring PCr recovery rates in three skeletal muscle groups, including the forearm ($N = 20$), upper leg ($N = 57$), and lower leg ($N = 51$) muscles, in both healthy and diseased individuals. All included studies have addressed different scientific questions using different experimental setups with specific requirements for data quality, such as duration of rest–exercise–recovery prescription, end-of-exercise pH, repetition time, the number of signal averages, temporal resolution, field strength, and coil size. These parameters varied significantly across studies, resulting in substantial variability in PCr recovery rates observed both within and between the three different skeletal muscle groups. This variability appears to be driven by methodological and/or physiological heterogeneity [132, 155]. Adequate values of these parameters and localization, as suggested in references [132, 161], can help to considerably improve upon the PCr recovery rate measures. In spite of this interstudy heterogeneity, the statistical analysis revealed similar pooled mean PCr recovery rates across all three muscle groups within studies that recruited solely healthy adults, but varied pooled mean PCr recovery rates were observed among the control and diseased groups of comparative studies across all muscle groups. The key implication of this finding is that there is a need to better understand other methodological and physiological causes of interstudy variability, for the purpose of standardizing measurement methodology across studies.

Meta-analysis revealed moderate overall heterogeneity in the reported PCr recovery rates of included studies. Variability in these measurements can be attributed to several factors, including variations in the physical fitness of the muscle, the recruitment and activation of similar or distinct muscle fibers [157, 162],

differences in exercise interventions and imaging protocols, and the effects of intracellular acidosis. This variation manifests itself as a broad range of measured values within even seemingly homogeneous participant groups. The results of this review suggest that this variability does not contribute substantially to the intermuscle group normal range differences within studies of healthy individuals, whereas it does contribute to such differences within control and diseased groups in comparative studies. These results emphasize the need to carefully select and validate the exercise protocol and ^{31}P-MRS method used to assess the PCr recovery kinetics for studying muscle PCr kinetics, especially in comparative studies. In particular, whereas there is a continual need to advance PCr recovery rate measurement methodologies in novel directions, building a coherent body of knowledge about skeletal muscle metabolism in these different participant groups will require greater standardization of experimental protocol across sites as suggested in reference [161], thus leading to greater consensus in corresponding measurement values across studies.

The macroscopic heterogeneity between skeletal muscles has also been recognized in humans [163–165]. In addition, the ^{31}P-MRS studies predominantly involve the use of surface coils for better signal-to-noise performance. Because the sensitivity of the surface coil decreases significantly as the distance from the coil increases, the use of superficial flexor muscles adjacent to the coil is generally preferred as tissue under investigation in these studies. As such, ^{31}P-MRS employing surface coil localization and voluntary exercise has long been a noninvasive method of choice for assessing human skeletal muscle function. However, it has never been considered a true correlation of mechanical and metabolic data from a single flexor muscle. In fact, because of the size and nonuniform dimensions of different flexor muscles, the measured data are a mixture of more than one muscle underlying the sensitivity profile of the surface coil [165]. The perfect example of this is the studies involving the forearm muscle group, which has a small heterogenous mixture of muscles under the volume of interest, which results in larger variations in PCr recovery kinetics. Thus, the size of the surface coil and its placement on the muscle affect the coverage of the tissue (intramuscle or intermuscle inclusion) under investigation and are expected to add to the PCr recovery rate's heterogeneity. Because the forearm muscles are small compared to the upper and lower legs, there is a greater chance of surface coil misplacement in the region of interest and/or combined data acquisition from multiple muscles in the forearm. This might result in larger variations in PCr kinetics in forearm muscles compared to upper or lower leg muscles (Figure 3). Additionally, as the forearm muscle studies have end-of-exercise pH in the lower range (6.2–6.9) (see Tables S1–S6) and the observation window for PCr recovery is much longer (15–20 min) compared to upper (5–6 min) and lower (5–6 min) leg muscles (see Tables S8–S13), this might greatly influence the PCr resynthesis rate in this muscle group. The mean PCr recovery time difference between control and disease groups in the upper leg was observed to be smaller compared to the forearm and lower leg muscle groups. We attribute this to the difference in the physiology and size of the muscle under investigation among different muscle groups. The quadriceps are known to have lower oxidative capacity and lower citrate synthase activity [157, 166] among the locomotor muscles, which might be the reason that they get less affected by the different diseases. Also, because the exercise protocols applied in upper leg studies involved placing the entire volume of interest (VOI) in a single large muscle, their results might have less variability compared to studies involving the forearm or lower leg muscle groups, which involve placing the VOI in a heterogeneous mixture of small muscles. Therefore, the results of upper leg studies lie in a narrow range and might more accurately represent the energetics of the involved muscle compared to the forearm or lower leg studies.

Although differing muscle-related diseases may be driven by differing underlying biology, common pathways involve muscle weakness, fatigue, reduction in muscle mass, and exercise intolerance leading to skeletal muscle impairment. Accordingly, in this review, the mean PCr recovery rate for individuals with muscle-related diseases consistently exceeded that of control groups in comparative studies, as well as that of participants in standalone studies of healthy adults, across all muscle groups regardless of the specific disease. This finding underscores the substantial impact that diverse diseases have in significantly modifying PCr recovery kinetics. Effect sizes for disease control group differences were largest in the forearm, then smaller in the lower leg, and then lower still in the upper leg. The reasons for intermuscle group differences in effect sizes are not clear.

A noteworthy finding in the violin plots (Figure 3) was significant differences in PCr recovery rate between healthy individuals who were recruited for studies that featured solely healthy individuals and the nominally healthy comparator groups recruited into studies of muscle-related diseases. We speculate that differences in the recruitment methods utilized in these two types of studies may account for this difference. Specifically, studies of diseases may have recruited controls that were matched with the disease group in terms of disease comorbidities that led to a poorer PCr recovery rate. In addition, studies featuring matched disease and control groups may have recruited control groups from hospitals or other health care settings similar to the settings where disease groups are readily recruited. Individuals recruited from health care settings may have been present in those settings because they featured poorer health generally—including poorer muscular health—than healthy individuals recruited from other settings (such as universities or athletic facilities) where healthy-only studies recruit readily. Future work should explore the effects that recruitment methods have on PCr recovery rate data among nominally healthy groups. In addition, future studies should place a greater emphasis on clearly reporting the specifics of recruitment methods for each participant group; indeed, we are limited to speculation about the effects of recruitment methods because published information about them is usually exceptionally terse.

The reduced heterogeneity in PCr recovery rates observed among truly healthy volunteers compared to the disease and control groups may partially result from methodological factors. Specifically, variations in sampling points, temporal resolution, and recovery period could have a greater impact on groups with comorbidities and diseases with slower recovery kinetics. A data acquisition duration that is appropriate for healthy populations but is too short for diseased populations could lead to an unstable estimation of the PCr recovery curve in the diseased populations due to the lack of return to baseline. Similarly, parameters such as the number of sampling points and temporal resolution

can also affect variability in the exponential data fitting process differently in groups with differing PCr recovery time characteristics. Nonetheless, physiologic differences, lifestyle factors, and the presence of comorbidities in the latter groups likely play a significant role in contributing to this variability. These factors highlight the complexity involved in interpreting PCr recovery kinetics across populations with diverse health conditions. Comorbidities, such as metabolic or age-related disorders, cardiovascular diseases, and chronic systemic inflammatory conditions, are known to influence mitochondrial function and could contribute to variability in PCr recovery kinetics between disease control groups. Although healthy volunteers were selected to minimize comorbidities, the disease control groups were likely to have a higher prevalence of these conditions with varying severity and duration. This difference may amplify observed group effects or obscure disease-specific contributions. Future studies should systematically assess the comorbid status and adjust for these variables to refine group comparisons and better isolate disease-specific mechanisms. This represents a limitation of the current study. Significant intermuscle group differences were found in end-of-exercise pH, with forearm muscle studies consistently reporting end-of-exercise pH levels ranging from 6.2 to 6.6, and leg muscle studies predominantly reporting end-of-exercise pH levels exceeding 6.9. We also report significant correlations between end-of-exercise pH and the PCr recovery rate in healthy individuals. In contrast, these correlations are observed to be nonsignificant in the control and disease groups. We speculate these differences are due to the presence of comorbidities in the control group, and the presence of muscular disorders in the disease group. The presence of any pathophysiology might impair muscle performance, which changes its relationship with pH during external stimuli like exercise [42]. The extent of intracellular acidosis reached at the end of exercise determines the rate of PCr resynthesis [16, 33, 157, 167]. In general, the process of PCr resynthesis involves both fast and slow components. When the contraction is intense and pH significantly changes ($pH << 6.9$), there appears to be an initial fast phase of PCr resynthesis followed by a second slower phase of PCr recovery. In contrast, low-intensity contraction does not involve significant pH changes ($pH \geq 6.9$) and can be best described by only the fast phase of PCr resynthesis. The initial rapid phase, influenced by ADP levels, occurs independently of muscle pH, whereas the secondary slower phase is rate-dependent on the return of the muscle cell to physiological pH ($pH \geq 6.9$) [155]. Thus, muscles with end-of-exercise $pH \geq 6.9$ are expected to resynthesize PCr faster than muscles with end-of-exercise $pH < 6.9$, which is consistent with our violin plot results for different muscles. The results of our correlation analysis are aligned with this understanding of PCr resynthesis and emphasize the need to tightly control pH as a means to reduce confounding of the PCr recovery rate by pH. Intermuscle group differences in end-of-exercise pH also suggest that this variable needs to be considered very carefully during any attempt to assess intermuscle group differences in PCr recovery kinetics from the current corpus of published studies [155, 161].

Among healthy adults, we found a moderately positive correlation between greater age and slower PCr recovery rate within forearm and upper leg muscles, with statistical significance noted only in the upper leg muscle group. Conversely, this relationship was negative, weak, and nonsignificant for the lower leg muscle group. We speculate that this lack of significant correlation between age and PCr recovery in the forearm and lower leg muscle groups in healthy individuals is due to the smaller number of studies within muscle groups (forearm, 8, and lower leg, 26), and the narrower range of ages (forearm, 10–40 years, and lower leg, 20–40 years) in those studies, both of which limited statistical power to detect significant correlations. Larger studies in these muscles with wider age ranges are required to address this correlation more fully. In the control and disease groups, the correlation was negative and nonsignificant for the forearm and lower leg muscle groups. However, in the upper leg muscle group, a moderately positive and significant correlation was found. Therefore, the conventional assumption that PCr recovery rate simply increases with age, in all populations and all parts of the body equally, should be assessed critically, and biological drivers of age-related increases in PCr recovery rate should be explored carefully.

5 | Conclusions

In summary, the current analysis of ^{31}P-MRS literature suggests that the mitochondrial oxidative capacity, as indicated by PCr recovery rate, is comparable across the three distinct skeletal muscle groups in healthy individuals. Furthermore, PCr recovery rates in skeletal muscles are consistently elevated in patients with diverse diseases compared to controls. It is important to acknowledge that our findings are subject to limitations stemming from variations in ^{31}P-MRS exercise protocols, parameters, and the restricted sample sizes in the included studies. Additional investigations addressing these limitations are warranted to validate our findings.

Another limitation of this study is the grouping of diseases based on their shared biological pathway of mitochondrial dysfunction. This grouping of multiple diseases into a single analysis was required because the number of studies available for each individual disease was insufficient for separate analyses. This approach limits our ability to make specific statements about individual diseases.

Methodological sources of heterogeneity in PCr recovery time measurements were identified through statistical analyses. Key factors include the exercise protocol, which affects muscle recruitment; coil size and placement, which influence whether data originate from superficial or deep muscles; and the number of sampling points, temporal resolution, and recovery period duration, all of which are crucial for accurately mapping PCr recovery to steady state and avoiding data truncation or bias during exponential recovery curve fitting. These factors significantly impact the reliability of PCr recovery measurements and must be carefully addressed in future studies.

Author Contributions

M.S. wrote the manuscript with comments from A.J., R.P., and O.C. All authors read and approved the final manuscript.

Data Availability Statement

The data supporting the findings of this systematic review are available within the article and its supplementary materials. All relevant data,

including search strategies, study selection criteria, and extracted information, have been included. Additional datasets generated or analyzed during the current study, such as detailed extraction tables and statistical analyses, are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.