PPP final report
PPPs that have been finalized need to deliver a factual and financial final report. For the financial report an overview of the project expenses on realisation and financing should be given in a separate format.
Final reports will be published in their entirety on the TKI/top-sector websites. Please make sure there are no confidential matters in the report.
PPP final reports have to be submitted - pooled for each research organisation - before 1 April 2019 to the TKIs at info@tkitu.nl, or at info@tki-agrifood.nl.

General data	
PPP number	AF-14503
Title	Active aging and dietary protein
Theme	Voeding en Gezondheid
Research Institute(s) involved	Maastricht University
Project leader research (name + email address)	L. van Loon (l.vanloon@maastrichtuniversity.nl)
Coordinator (on behalf of private parties)	
Contact person of government	
Total project budget (k€)	692
Project website address	www.tifn.nl
Starting date	1-1-2014
Final date	31-12-2017

Approval coordinator/consortium The final report has to be discussed with the coordinator/consortium. The TKI(s) like to be informed regarding potential comments on the final report.	
The annual report is …… by the coordinator on behalf of the consortium	□ approved □ not approved
Potential comments regarding the final report	--

Brief description content/aim PPP What is the matter and what does the project contribute? What does the project deliver and what are the effects of its delivery?
To define the optimal amount of intrinsically labelled dietary protein to maximize the post-exercise muscle protein synthetic response in the older population. To define the relevance for post-exercise co-ingestion of free leucine with protein to reduce the total amount of dietary protein required to maximize the post-prandial muscle protein synthetic response following exercise in the older population. To provide evidence for the efficacy of protein (with or without free leucine) supplementation to augment the gains in muscle mass and strength following prolonged exercise training in the older population. The project was hosted as an extension of a large TiFN project on Muscle health and function.

Mutations with respect to the original project plan and follow-up	
Have there been changes in the consortium/project partners? If so, which.	No

Have there been factual changes in the project?	No
Has a patent application been filed from this PPP (or a priority filing)?	No
Has a spin-off developed from this project (contract research, additional funding or spin-off activity)?	No
How many years will the private parties need in practice to use results from this project?	Not applicable
How did the project contribute to the development of the research organisation involved (e.g. scientific track record, new technology, new collaboration)?	Not applicable
Will there be a follow-up for the project such as a new project or a new collaboration? If so, please explain.	Not applicable

Results

What tangible results the project has yielded?

Large groups of elderly have been trained in this project. They were very enthusiastic. Physically a lot of imrpovements on health, body composition and strength were noticed. The effect of extra protein was not detected. The training did have clear results.

What are the effects of these results and for whom?

See answer to question above.

What has not been delivered according to the original project plan and for what reason(s)?

Not applicable.

Deliverables (give a short description per project deliverable)

Study 1

Objective: To define the optimal amount of intrinsically labeled dietary protein to maximize the post-exercise muscle protein synthetic response in the older population.

Deliverables: Definition of the dose response of the amount of dairy protein ingestion following exercise on myofibrillar muscle protein synthesis rates in the older population.

Design: 36 healthy, lean elderly males (age: 55-80 y) will be selected to participate in this study. Subjects will be screened with medical questionnaires, habitual food and physical activity diaries, body composition (DEXA and anthropometric evaluation), resting ECG, overall functional capacity. Upon inclusion, subjects will participate in an experimental trial during which they will ingest 0, 15, 30 or 45 g dairy protein. Contemporary stable isotope methodology will be applied with continuous intravenous infusion of [ring-$^{2}H_5$] phenylalanine, [1-^{13}C] leucine and [3,5-$^{2}H_2$] tyrosine to allow assessment of mixed and myofibrillar muscle protein synthesis rates, whole body protein kinetics and digestion and absorption rates as well as the amount of dietary protein-derived phenylalanine that will be incorporated into skeletal muscle. Blood samples are collected frequently during the infusion period (t = -150, -120, -60, 0, 30, 60, 90, 120, 180, 240, 300, 360 min.) and muscle biopsy samples will be taken from the *vastus lateralis* muscle at t = 0 hours and 6 hours after protein ingestion.

Analyses: Plasma glucose, insulin, leucine, phenylalanine, tyrosine concentrations; plasma, muscle free, and muscle protein bound [ring-$^{2}H_5$] phenylalanine and [1-^{13}C]-leucine enrichments, and plasma [3,5-$^{2}H_2$] tyrosine enrichments.

Study 2
Objective: To define the relevance for post-exercise co-ingestion of free leucine with protein to reduce the total amount of dietary protein required to maximize the post-prandial muscle protein synthetic rate following exercise in the older population.
Deliverables: Definition of the capacity of co-ingesting free leucine with protein to further increase post-prandial muscle protein synthesis rate following exercise in older men.
Design: 24 healthy, lean elderly males (age: 55-80 yrs.) will be selected to participate in this study. Subjects will be screened with medical questionnaires, habitual food and physical activity diaries, body composition (DEXA and anthropometic evaluation), resting ECG, overall functional capacity. Upon inclusion, subjects will participate in an experimental trial during which they will ingest 15 g dairy protein with or without 1.5 g free leucine. Contemporary stable isotope methodology will be applied with continuous intravenous infusion of [ring-$^{2}H_5$] phenylalanine, [1-^{13}C] leucine and [3,5-$^{2}H_2$] tyrosine to allow assessment of mixed and myofibrillar muscle protein synthesis rates, whole body protein kinetics and digestion and absorption rates as well as the amount of dietary protein-derived phenylalanine that will be incorporated into skeletal muscle. Blood samples are collected frequently during the infusion period (t = -150, -120, -60, 0, 30, 60, 90, 120, 180, 240, 300, 360 min) and muscle biopsy samples will be taken from the *vastus lateralis* muscle at t = 0 hours and 6 hours after protein ingestion.
Analyses: Plasma glucose, insulin, leucine, phenylalanine, tyrosine concentrations; plasma, muscle free, and muscle protein bound [ring-$^{2}H_5$] phenylalanine and [1-^{13}C]-leucine enrichments, and plasma [3,5-$^{2}H_2$] tyrosine enrichments.

Study 3
Objective: To provide evidence for the efficacy of protein - with or without free leucine - supplementation to augment the gains in muscle mass and strength following prolonged exercise training in the older population.
Deliverables: Definition of the capacity of protein supplementation to augment the gains in muscle mass and strength during prolonged exercise training in the elderly population.
Design: 40 healthy, lean elderly males (age: 55-80 yrs.) will be selected to participate in this study. Subjects will be screened with medical questionnaires, habitual food and physical activity diaries, body composition (DEXA and anthropometric evaluation), resting ECG, overall functional capacity. Upon inclusion, subjects will participate in an 3 month exercise training program. One group will be supplemented with 15 g dairy protein (with or without free leucine based upon the outcome of studies 1-2) after each exercise session (2-3 per week) and 15 g prior to sleep. The other groups will receive an isocaloric placebo (without protein). Prior to and after the 3 month intervention body composition (DEXA, CT), strength (1-RM), and functional capacity (SPPB) will be assessed, and blood and muscle biopsy samples will be collected. In case of additional funding available, a post-prandial muscle protein synthetic response could be performed prior to and after the exercise training programme.
Analyses: Plasma glucose, insulin, leucine, phenylalanine, tyrosine, myostatin concentrations, whole-body fat and fat free mass (DEXA), leg muscle cross sectional area (CT), muscle strength (1-RM), functional capacity (SPPB), muscle fiber characteristics (immune-histochemical analyses), associated signaling (Western blotting).

The extra contribution will allow us to use intrinsically labeled protein to be able to assess whole body kinetics and digestion and absorption data as well as the amount of dietary protein-derived phenylalanine that will be incorporated into skeletal muscle. All subject groups will ingest different amounts of this intrinsically [1-13C] Phe and [1-13C] Leu labeled protein drink. They will get a continuous intravenous infusion of [ring-2H5] Phe, [3,5-2H2] Tyr and [1-13C] Leu instead of the [U-13C] Phe and [6,6-2H2] Tyr now described in the proposal.

Number of delivered products (give titles and/or descriptions of products, or a link to the products on the project website, or other public websites).			
Scientific articles and PhD thesis	Reports	Articles in professional journals	Lectures/workshops/ posters
8			14

Annex: Titles of deliverables or a link to products on the project website or other public websites

1. Scientific articles

- Holwerda, A.M., K. Lenaerts, J. Bierau and L.C.J. van Loon (2016). Body position modulates gastric emptying and affects the postprandial rise in amino acid concentrations following protein ingestion in humans. Nutr. 8: 221.

- Holwerda, A.M., I.W.K. Kouw, J. Trommelen, S.L. Halson, W.K.W.H. Wodzig, L.B. Verdijk and L.C.J. van Loon (2016). Physical activity performed in the evening increases the overnight muscle protein synthetic response to pre-sleep protein-ingestion in older men. J. Nutr. 146: 1307 - 1314.

- Holwerda, A.M., K. Lenaerts, J. Bierau, W.K.W.H. Wodzig and L.J.C. van Loon (2017). Food ingestion in an upright sitting position increases postprandial amino acid availability when compared with food ingestion in a lying down position. Appl. Physiol. Nutr. Metab. 42: 738 - 743.

- Holwerda, A.M., K.J.M. Paulussen, M. Overkamp, J.S.J. Smeets, A.P. Gijsen, J.P.B. Goessens, L.B. Verdijk and L.J.C. van Loon (2018). Daily resistance-type exercise stimulates muscle protein synthesis in vivo in young men. J. Appl. Physiol. 124: 66 - 75.

- Holwerda, A.M., M. Overkamp, K.J.M. Paulussen, J.S.J. Smeets, J. van Kranenburg, E.M.P. Backx, A.P. Gijsen, J.P.B. Goessens, L.B. Verdijk and L.J.C. van Loon (2018). Protein supplementation after exercise and before sleep does not further augment muscle mass and strength gains during resistance exercise training in active older men. J. Nutr. 148: 1723 - 1732; doi: 10.1093/jn/nxy169.

- Holwerda, A.M., K.J.M. Paulussen, M. Overkamp, J.P.B. Goessens, I.-F. Kramer, W.K.W.H. Wodzig, L.B. Verdijk and L.J.C. van Loon (2019). Dose-dependent increases in whole-body net protein balance and dietary protein-derived amino acid incorporation into myofibrillar protein during recovery from resistance exercise. J. Nutr., doi: 10.1093/jn/nxy263.

- Holwerda, A.M. (2019). Dietary protein to support active aging. PhD thesis, Maastricht University.

- Holwerda, A.M., K.J.M. Paulussen, M. Overkamp, J.P.B. Goessens, I.-F. Kramer, W.K.W.H. Wodzig, L.B.Verdijk, L.C.P.G.M. de Groot and L.C.J. van Loon. Leucine co-ingestion augments the muscle protein synthetic response to ingestion of 15 g protein following resistance exercise in older men (submitted).

2. Lectures

- Holwerda A.M., I.W. Kouw, J. Trommelen, S.L. Halson, W.K.W.H. Wodzig, L.B. Verdijk and L.J.C. van Loon, Exercise enhances the overnight muscle protein synthetic response to pre-sleep protein feeding in older males. European College of Sport Science (ECSS) Annual Congress, July 2016, Vienna, Austria.

- Holwerda, A.M., K.J.M. Paulussen, M. Overkamp, J.P.B. Goessens, A.-F. Kramer, W.K.W.H. Wodzig, L.B. Verdijk and L.J.C. van Loon, Postprandial protein handling following ingestion of different amounts of protein during post-exercise recovery in older males. Experimental Biology Annual Meeting, April 2017, Chicago, USA; in Benelux Association of Stable Isotope Scientists (BASIS) Annual meeting, May 2017, Utrecht, the Netherlands; in European College of Sport Science (ECSS) Annual Congress, July 2017, Metropolis-Ruhr, Germany.

- Holwerda, A.M., Latest research insights into protein timing–window of opportunity in the evening. Bridge2Food: 7th Sports & Performance Nutrition Summit, June 2017, Amsterdam, the Netherlands.

- Holwerda, A.M., Exercise and dietary protein to support healthy ageing. Dept. Health and Exercise Science, Colorado State University, August 2017, Fort Collins, USA.

- Holwerda, A.H., Deuterium oxide for the measurement of muscle protein synthesis in humans. Masterclass: The use of stable isotopes in clinical research. September 2017, Maastricht University, Maastricht, the Netherlands.

- Holwerda, A.M., The use of labelled water to assess muscle protein synthesis rates. ESPEN intensive course in tracer methodology in metabolism. June 2018, Maastricht University, Maastricht, the Netherlands.

- Holwerda, A.M., M. Overkamp, K.J.M. Paulussen, J.S.J. Smeets, A.P. Gijsen, J.P.B. Goessens, L.B. Verdijk and L.C.J. van Loon, Leucine-enriched protein supplementation does not augment muscle mass and strength gains during resistance-type exercise training in older males. European College of Sport Science (ECSS) Annual Congress, July 2018, Dublin, Ireland.

3. Posters

- Holwerda, A.M., K. Lenaerts, J. Bierau and L.J.C. van Loon, Gastric emptying is delayed when protein is ingested in a supine compared with a sitting position. NUTRIM Symposium, December 2015, Maastricht University, Maastricht, the Netherlands.

- Holwerda, A.M., I.W. Kouw, J. Trommelen, S.L. Halson, W.K.W.H. Wodzig, L.B. Verdijk and L.J.C. van Loon, Physical activity performed in the evening increases the overnight muscle protein synthetic response to pre-sleep protein feeding in older men. American College of Sports Medicine Annual Meeting, May 2016, Boston, USA.

- Holwerda, A.M., A.P. Gijsen, J.P.B. Goessens, J.M.G. Senden, D. de Boer, W.K.W.H. Wodzig and L.J.C. van Loon, Deuterium oxide dosing to measure muscle protein synthesis rates in vivo in humans. Joint European Stable Isotopes User group Meeting (JESIUM), September 2016, Ghent, Belgium.

- Holwerda, A.M., K.J.M. Paulussen, M. Overkamp, J.P.B. Goessens, A.-F. Kramer, W.K.W.H. Wodzig, L.B. Verdijk and L.J.C. van Loon, Postprandial protein handling following ingestion of different amounts of protein during post-exercise recovery in older males. Dutch Society for Research on Ageing (DuSRA) Annual Meeting, May 2017, Leiden, the Netherlands.

- Holwerda, A.M., M. Overkamp, K.J.M. Paulussen, J.S.J. Smeets, A.P. Gijsen, J.P.B. Goessens, L.B. Verdijk and L.C.J. van Loon, Leucine-enriched protein supplementation does not augment muscle mass and strength gains during resistance-type exercise training in older males. American College of Sport Medicine Annual Meeting, May 2018, Minneapolis, USA.