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Original article

Supplemental parenteral nutrition improves immunity with unchanged carbohydrate and protein metabolism in critically ill patients: The SPN2 randomized tracer study

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SUMMARY

Background & aims: Individualized supplemental parenteral nutrition (SPN) providing measured energy expenditure from day 4 reduced infectious complications in a previous study including 305 intensive care (ICU) patients. The study aimed at investigating the metabolic, and immune responses underlying the clinical response of the previous trial.

Methods: Randomized controlled trial enrolling 23 critically ill patients on day 3 (D3) of admission to the ICU who were fed less than 60% of their energy target by the enteral nutrition (EN) alone: allocation to either continued EN or to SPN to a target validated by indirect calorimetry. Protein and glucose metabolism (primary endpoint) were investigated with tracer isotopes on D4 and D9. Secondary endpoints: 1) immune response, investigated in serum and in stimulated peripheral blood mononuclear cells (PMBC), by dosing a panel of cytokines (infectious complications were recorded), and 2) Muscle mass was assessed by ultrasound of the thigh.

Results: Comparable at baseline, the SPN group ($n = 11$) received more energy (median 24.3 versus 17.8 kcal/kg/day: $p < 0.001$) and proteins (1.11 versus 0.69 g/kg/day: $p < 0.001$) than the control group during the five days' intervention, resulting in a less negative energy balance by D9 ($p = 0.0027$). Net protein breakdown and Glucose kinetics on D9 did not differ, within or between groups. In agreement with a decrease in infection rate, immune response in the SPN group showed decreased serum IL-6 ($p = 0.024$), IL-1β, IL-10 levels and TNF-α secretion by PBMC ($p = 0.018$) at D9. Muscle mass loss from D4 to D15 tended to be less in the SPN group (−16% versus −23%: $p = 0.06$). Clinical course by D28 did not differ.

Conclusions: Feeding patients to cover an individualised measured energy target with SPN from D4 to cover needs, was associated with improved immunity, less systemic inflammation and a trend to less muscle mass loss.

Clinical trial registry: NCT02022813 at https://clinicaltrials.gov/

Abbreviations: ARDS, Acute respiratory distress syndrome; AUC, Area under the curve; CSA, Cross sectional areas; CRP, C-reactive protein; D, Day; EE, Energy expenditure; EN, Enteral nutrition; EGP, Endogenous glucose production; EPD, Endogenous protein degradation; Exo, Exogenous; GNG, Gluconeogenesis; GRa, Glucose rate of appearance; IC, Indirect calorimetry; IL, Interleukin; IQR, Interquartile ranges; Leu, Leucine; PN, Parenteral nutrition; PMBC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; Ra, Rate of appearance; R, Responders; SPN, Supplemental parenteral nutrition; TNF, Tumor necrosis factor.

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1. Introduction

The last decade has been marked by a debate regarding the optimal timing, as well as the quantities of energy and proteins to be provided during the first 7–10 days of critical illness. Among the randomised trials the Swiss supplemental parenteral nutrition trial (SPN-1 [1]) tested the completion of an insufficient enteral nutrition (EN), i.e. not reaching 60% of target, from day 4 (D4) on, and for five days with additional parenteral nutrition (PN) to an individually measured energy target. The results in 305 patients showed a reduction of infectious complications ($p = 0.04$), and costs (minus 3.300 CHF per case) [2]: the major drivers of the cost reduction were the improved energy balance, and the lesser costs associated with anti-infectious treatment.

Of note, the patients of the Swiss SPN-1 trial were a carefully selected homogeneous patient cohort enrolled on day 3 after intensive care unit (ICU) admission based on failure of EN to cover the nutritional needs, and their likely need for further five days of ICU treatment. Thereafter the nutritional treatment was carefully individualized with indirect calorimetry. Protein delivery was close to the target of 1.2 g/kg/day during the intervention [3]. Importantly, there was no attempt to compensate the patients' first 3 days' extrinsic energy deficit.

The mechanism underlying the better outcome in the Swiss SPN-1 study was hypothesized to be an improvement of immunity mediated by a lower energy deficit, and possibly also by a better utilisation of substrates. The medico-economic study had indeed shown that there was a 10% reduction in infection risk per 1000 kcal decrease in cumulative energy deficit [2]. Substrate handling (protein, and glucose) might have been modified, and immune response might have been modulated. While muscle mass decrease has been shown to be associated with worsening of outcome [4], no muscle mass data were available.

The present randomised trial aimed at investigating the potential mechanisms underlying the reduction of infectious complications observed in the SPN group of the initial trial [1] in a similarly selected study population, whose gut was not enabling feeding to measured energy target while requiring further ICU treatment.

2. Patients and methods

2.1. Study design

We conducted a randomized, prospective, open, controlled study in the 35 bed multidisciplinary ICU of the Lausanne University hospital (NCT02022813), replicating the conditions of the Swiss SPN-1 trial (Fig. 1A). Ethical approval was obtained from the Commission cantonale d'éthique de la recherche sur l'être humain (CER-VD 371-13), and written informed consent was obtained from the patients, or their legal representatives.

2.2. Study population

Inclusion criteria were as in the previous study [1]: mechanically ventilated patients with a functional gut, who by end of day 3 did not receive 60% of the equation target (25 kcal/kg*day) by EN alone, and who were expected to require a further 5 days of ICU therapy with full treatment. Exclusion criteria were the same as in SPN-1 [1] (see web-appendix), with the additional exclusion of severe brain injury, and cardiac arrest because of likely metabolic difference, and absence of endotracheal intubation (non-invasive ventilation was accepted in SPN-1) to ensure a precise indirect calorimetry (IC) determination of energy goals.

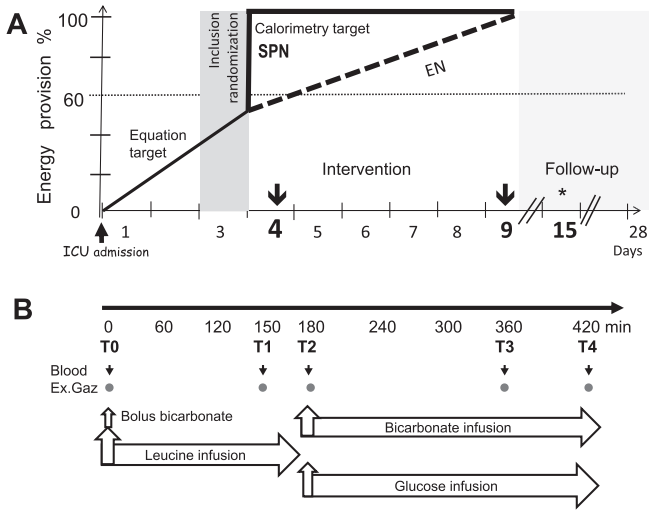

Fig. 1. Study design: Panel A shows the global design. Indirect calorimetry was performed on days 4 and 9, while ultrasound of the thigh was repeated on days 4, 9 and 15. Panel B shows the detail of the glucose and leucine tracer studies on days 4 and 9: The vertical empty arrows indicate a bolus injection of tracer (leucine, glucose or bicarbonate.

Randomisation and masking: On day 3, consecutive patients were randomized to receive EN with SPN, or to EN alone. A computer-generated randomisation sequence was used, that included stratification by gender, and admission category (surgery, medicine) with a block size of two for SPN, or EN. Allocation concealment was achieved with sequentially numbered, sealed, opaque envelopes. The daily on-duty investigator told the physician in charge which treatment had been assigned. The study was open as the nutritional treatment could not be hidden (care providers, and patients were not masked); however, the investigators who established caloric goals were not directly involved in patient care. The immunologists, assessors, and statisticians were blinded to group allocation.

For all clinical aspects, the protocol replicated the methods of SPN-1 (detailed in web-appendix including nutrition protocol): the additional investigations are indicated hereafter (Fig. 1B). Continuous insulin infusion was used to maintain blood glucose within 6–8 mmol. Area under the curve (AUC) of all arterial blood glucose values was calculated.

2.3. Protein turnover

The measurements were performed from time 0 (T0) to 150 min (T1). They were based on the analysis of isotope dilution of ^{13}C-leucine, an amino-acid representative of global protein metabolism, and of its oxidation to $^{13}CO_2$ (see web-appendix). Briefly a bolus of $NaH^{13}CO_3$ (0.01 mmol) and of L-[1–^{13}C]-Leucine (5 mmol/kg) was administered intravenously on a central venous line (jugular or subclavian) at T0. The bolus of bicarbonate aimed at speeding up the enrichment in breath $^{13}CO_2$. The bolus was followed by a continuous infusion of L-[1–^{13}C]-Leucine at a constant rate of 0.05 mmol/kg/min from time T0 to T180. Blood samples were collected from an arterial line (radial or femoral) at times T0, 150 and 180. Blood samples were collected at times T0, 150 and 180 for measurement of ^{13}C isotope enrichment of blood leucine and of keto-isocaproic acid (KIC); breath samples were collected at the same time points for measurement of $^{13}CO_2$ enrichment. The total protein synthesis was calculated as being equal to ((rate of appearance [Ra] leucine) - (Oxidation leucine))* 1/0.08. This calculation was made by estimating that leucine contributes to 8%

of the amino acids of proteins [5]. Endogenous protein degradation (EPD) was calculated as = ((Leu Ra − Ra exogenous leucine) * 1/0.08. The rate of appearance of exogenous leucine could not be directly measured due to multiple (enteral protein, and iv amino acids) sources of leucine intake, and was assumed to be equal to the sum of the rates of leucine administered enterally, and intravenously (i.e. assuming complete absorption of leucine from enterally infused proteins).

2.4. Glucose kinetics

The calculations were based on the simultaneous measurement of total glucose rate of appearance (GRa) by isotope dilution analysis of infused $^{2}H_2$ glucose with primed-continuous infusion of 6,6 $^{2}H_2$ glucose, and of $^{13}CO_2$ incorporation in arterial blood glucose; for this purpose, whole body CO_2 pool was labelled with a constant infusion of ^{13}C-labelled bicarbonate. The GRa was calculated from plasma glucose 6,6 $^{2}H_2$ isotopic enrichment with Steele's equations for non-steady state [6] (see web-appendix). At the end of ^{13}C-leucine infusion (T2), ^{13}C bicarbonate was infused to enrich blood bicarbonate in the 0-5-1% range, and incorporation of ^{13}C in plasma glucose (which takes place at the level of pyruvate carboxylase) was used to calculate fractional gluconeogenesis (from pyruvate, lactate, and pyruvate-yielding amino acids) [7]. Gluconeogenesis (GNG), estimated from ^{13}C-glucose synthesis during continuous ^{13}C-bicarbonate infusion, was calculated as [8]: = (^{13}C-glucose * 6)/($^{13}CO_2$ * GRa), where ^{13}C-glucose, and $^{13}CO_2$ are isotopic enrichments in Atom Percent Excess.

2.5. Immunity, inflammation and infectious complications

Cell stimulation: After thawing, the peripheral blood mononuclear cells (PBMC) were washed and distributed in 96-round-bottom well plates ($2{\cdot}5 \times 10^5$ cells/well) in 0·2 ml RPMI medium (Sigma Chemical Co, St. Louis, MO, USA). RPMI was supplemented with glutamine (Sigma), 100 IU/mL penicillin-streptomycin, 100 μM non-essential amino acids, kanamycin, 2 mM sodium pyruvate (Invitrogen), 8% human AB serum (AB) (Blutspendedienst SRK Bern AG, CH-Bern). Cells were stimulated in 5-plicates for 6 days with recall antigens (tetanus toxoid, candida PPD, or for 2 days with phytohemagglutinin = PHA), or left unstimulated as control. Proliferation was assessed with addition of tritiated thymidine. Responders have a minimum increase of 20% of their proliferative response to PHA.

Cytokine measurement: IL-1β, IL-2, IL-5, IL-6, IL-8, IL-10, IL-13, TNFα, and IFNγ were measured in serum, or in culture supernatants after two-day or six-days stimulation with recall antigens by multiplexed particle-based flow cytometric assays (Bioplex, Bio-Rad Laboratories, Hercules, CA, USA) according to manufacturer's instructions, and acquisition performed using a Luminex 100. Results are expressed in pg/mL.

Infectious complications were collected prospectively respecting the definitions of the 2005 consensus conference [9].

2.6. Muscle ultrasound and other laboratory variables

Muscle mass was investigated using the panoramic brightness-mode ultrasound determination [10] of the cross-sectional area (CSA) of the quadriceps femoris [11] on days 4, 9–10, and 15. A custom harness was fixed on the patient's thigh to ensure fixed distances between the base of the patella, and the level of muscle measurement.

Laboratory determinations: Transthyretin, and C-reactive proteins were determined by standard clinical chemistry methods.

2.7. Sample size and statistical analysis

Sample size: A sample size calculation was not done. As published by others [12], the available knowledge in the domain of protein remains limited. The physiological trials have included 6–10 patients per group in the past: based on our group's studies [13], where two times eight patients had proven sufficient to show differences, a “probably safe” number of patients enabling detection of differences was decided to be two times 14 patients.

Statistical analysis: Variables are presented as median, and interquartile ranges (Q1, Q3). The questions addressed were statistically analysed separately. We did descriptive analyses with the χ^2, or Fisher's exact tests for categorical variables, and the Student's *t* test, or Mann-Whitney-Wilcoxon test for continuous variables when appropriate. Changes over time between groups, as well as within groups were analyzed. Significance was considered at the level of $p < 0.05$. Trends were considered for $p \leq 0.2$. The statistical package was JMP ® Version 10.0, SAS Institute INC: Cary, NC, USA.

3. Results

3.1. Patients

Altogether 28 patients (of 862 screened) were enrolled, and 23 completed the study, i.e. completed the 2nd tracer course (Consort diagram Fig. 2) between May 2014 and April 2016. There was no significant difference between the two groups (Table 1) or with the previous SPN-1 cohort (Table 1 web appendix).

3.2. Energy and nutrition

Energy: Energy expenditure (EE) on day 3 became the first intervention energy target (Table 1); the prescription was adapted to the new value. Energy delivery increased during day 3 in the SPN patients and was at target between D4 and day 9 (D9) (Fig. 3). While both groups started D4 with a similar energy deficit, the SPN group was at equilibrium during the intervention with no catch up of deficit, compared to the continued progression of deficit in the EN group (Table 2). The cumulated energy balances at D9 were significantly less negative in the SPN group (2614 kcal less in the SPN group: $p = 0.0027$).

Protein intakes were close to target with 1.16 g/kg/day in the SPN group (Fig. 3), and nearly double of those of the EN group.

Total glucose intake per day was higher in the SPN group (2.74 versus 2.1 g/kg/day: $p < 0.0001$). Plasma glucose during the study was stable, and similar in both groups. The AUC of glycemia during the 5 days did not differ between groups. Insulin requirements were higher in the SPN patients during the 5 days, and during the 2 tracer days (67.6 u/24h versus 34.8 u/24 h: $p = 0.0015$). Two patients in each group were insulin-free on day 4.

3.3. Leucine turnover

Leucine Ra, and Leucine oxidation were similar in both groups on D4, both being significantly higher in the SPN group on D9 (Table 3A). Protein degradation, and total protein synthesis were higher after SPN than EN, but net protein breakdown (i.e. protein degradation minus total protein synthesis) were not significantly different from zero in both groups at D9, possibly reflecting a small difference in protein intakes on that day (Fig. 3). Transthyretin increased significantly faster in the SPN group (Fig. 4).

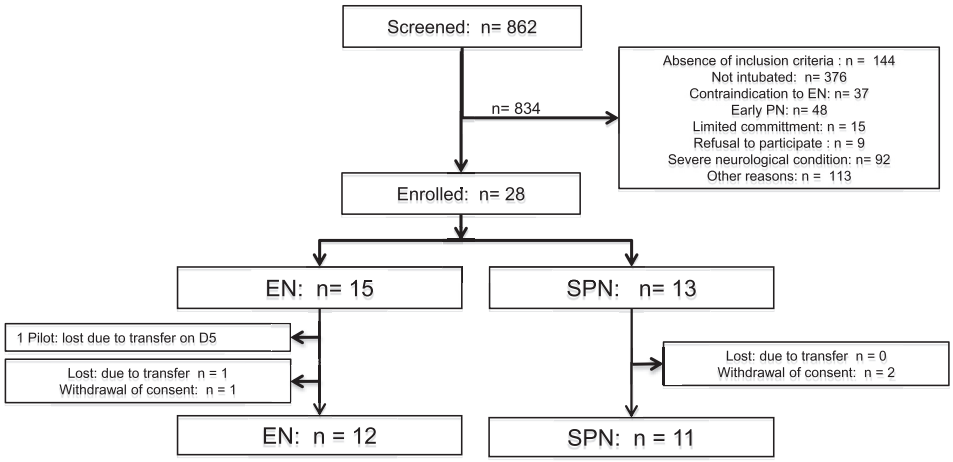

Fig. 2. Consort diagram.

Table 1
Patient demographic, and metabolic characteristics.

	Units	EN (n = 12)		SPN (n = 11)		p
Age	years	67.5	62.3–75	63	55–73	ns
Gender	M/F	10/2		9/2		ns
Weight	kg	77	75–90	79	69–98	ns
Ideal body weight	kg	66.5	59–74	66.0	64–73	ns
Height	cm	169	162–176	172	170–180	ns
Body mass index	kg/m^2	25.2	23.8–29.9	27.8	26.3–30.9	ns
Medical/surgical		7/5		3/8		0.13
APACHE II		23	19.2–27.8	25	17–26	ns
SAPS II		45.5	37.3–60	50	37–60	ns
SOFA – admission day		11	7–12	9	6–11	ns
SOFA – Day3		8	7–11	10	7–11	ns
Infection on admission	n	4/12		3/11		ns
Calculated Energy target	kcal/d	1788	1520–2006	1800	1500–1975	ns
MEE Day 03	kcal/kg/d	23.5	16.6–26.2	23.2	19.3–25.0	ns
MEE Day 04	kcal/kg/d	23.3	18.7–26.0	24.1	20.6–27.1	ns
MEE Day 09	kcal/kg/d	23.8	22.0–30.1	24.9	22.3–27.0	ns

Median (Q1-Q3 ranges).
MEE = measured energy expenditure, Ideal Body weight according to table from the ARDS net: https://mpog.org/files/quality/toolkit/ibw_tv_chart1.pdf.

3.4. Glucose kinetics

Glucose Ra was higher in the SPN group on days 4 and 9, reflecting the higher intakes in the SPN group (GRExo) (Table 3B). Endogenous glucose production (EGP) did not differ between groups on study days, despite modestly lower values in the SPN group on both days. The median EGP expressed in g/day on D4 was 182 g/day (all patients), 205 g in EN versus 155 g glucose in SPN ($p = 0.04$): on D9, EGP was 138.2 g/day (all), 135.3 g in EN, versus 127.4 g glucose in the SPN group (ns). Gluconeogenesis was lower than EGP on both days, and in both groups, being similar on D4, and modestly but non-significantly higher on D9 in the SPN group.

3.5. Immunity, inflammation and infectious complications

Systemic inflammatory markers: Serum levels of IL-1β, IL-6, IL-10, IL-8, and CRP were elevated at D4 in a majority of patients from both groups compared to levels in control sera, a situation compatible with acute inflammation. Serum IL-6 dropped significantly at D9 in the SPN group as compared to D4 ($p = 0.0244$): serum IL-6 was unchanged in the EN group (Fig. 5 panels A, B). The change in IL-6 over time tended to me more important in the SPN group ($p = 0.061$). A similar trend was present in the post/pre ratio for IL-1β (Fig. 5 panels D, E), being consistent with a decrease in serum IL-10 levels in the SPN versus EN group. CRP also decreased in both groups, tending to decrease more in the SPN group (Fig. 4). There was no significant change, or trends with other serum cytokines, including TNFα (Fig. 5B).

Cell mediated immunity: PBMC were stimulated with mitogen (phytohemagglutin, PHA). We observed a significant decrease in TNFα secretion into supernatant in the SPN group as compared to pre-treatment level ($p < 0.0186$), but not in the EN group (Fig. 5C). There was no significant change for IL-2, IFNγ, IL-5, IL-13, IL-10, IL-6, IL-1β, and IL-8 (Fig. 5D). A trend towards an enhanced proliferative capacity to PHA was observed in the SPN group as compared to the EN group (Fig. 5D). A similar trend in favour of an improved proliferative response to PHA was observed when expressed as number of responders in both groups (Fig. 5F). After PBMC stimulation with recall antigens (IFNγ, TNFα), two key Th1 cytokines of the anti-infectious response, were significantly decreased in PBMC from EN group, as was IL-8 (Fig. 5E).

Infections (Table 2): In the SPN group, the number of nosocomial infections after D9 was lower (median 0 versus 1) although not significantly, and antibiotic free days were not significantly higher (median 18 versus 10.5 days). Aggregation of the infectious complications of the two trials showed a reinforcement of the SPN-1 results mainly driven by the first trial (Fig. 1 web-appendix).

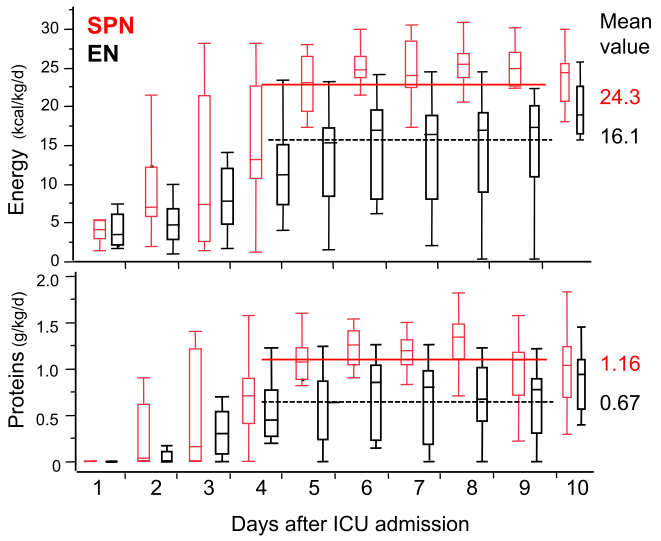

Fig. 3. Energy and Protein intakes before and during the 5 days-intervention. The horizontal red, and black lines represent the mean intake of each group during the 5 days intervention. While the change over time was significant in both groups ($p < 0.0001$), the difference between groups was $p = 0.006$ for energy, and $p = 0.05$ for proteins. The mean value of intakes during the intervention is indicated on the right side.

3.6. Muscle ultrasound (CSA)

For technical reasons, the three determinations (days 4, 9, and 15) were available in only 21 patients. All patients lost muscle mass, the decline becoming highly significant by day 15 ($p < 0.0001$) with a median CSA decrease of −20.4% from D4. While there was an important individual variation, the total loss of muscle surface tended to be less in the SPN group (−16% versus −23%: $p = 0.068$: Fig. 4). The inter- and intra-observer reliability were both excellent (variability<5%: ICCs = 0.950, and 0.979, respectively).

4. Discussion

The present study shows that in a selected population of critically ill patients the provision of energy, and proteins by means of a supplemental PN to cover an individually measured energy target improves immunity without being detrimental to either glucose, or protein metabolism. Despite covering energy needs, and recommended higher protein intakes in the SPN group, this did not translate into significant effects on protein synthesis, or breakdown. This tracer study was a crude, exploratory assessment of protein metabolism. Nevertheless, our observation that SPN significantly modified the immune function (see below) suggests that improvement of protein, and energy metabolism in discrete specific organs, and systems may have passed undetected.

Glucose kinetics was strictly similar in both groups despite a higher dose of insulin being needed in the SPN group to maintain

Table 2
Clinical outcome variables.

	Units	EN (n = 12)		SPN (n = 11)		p
Cumulated energy balance						
- ICU Admission to day 3	kcal/24h	−3448	−4063 to −2796	−2741	−3130 to −2406	0.0522
- Day 4–9	kcal/24h	−3018	−6671 to −1412	−90	−1503 to 47	0.0055
- ICU Admission to day 9	kcal/24h	−6050	−10′637 to −4830	−3416	−4027 to −2336	0.0027
Blood glucose days 4–9	mmo/l	7.3	5.7–8.6	7.5	5.9–8.4	ns
AUC glucose days 4–9	mmol/l	1091	977–1183	1062	1025–1185	ns
Insulin dose/24h days 4–9	IU/24h	8	0–125	48	19–179	0.0031
Length of mechanical ventilation	days	5.5	4.2–14.5	8.9	4.9–15.7	ns
Renal replacement therapy	n	3		3		ns
Length ICU stay	days	9.5	7.1–24.4	15.3	10.6–17.4	ns
Length hospital stay	days	48	25–59	44	30–57	ns
Hospital death	n	1		0		ns
Antibiotic prophylaxis	days	2.5	0–6	1	0–3	ns
Antibiotics total	days	13.5	7–17	8	3–15	ns
Antibiotic free days	days	10.5	6.3–17	18	12–20	0.2597
Infections →D28	n	1	1–2	1	1–1	ns
Nosocomial infections≥D3	n	1	0–2	0	0–1	0.1215
Time to 1st infection after D3	days	9	7.5–22	28*	10–28	0.132
Time to 1st infection after D9	days	13.5	9–28	28*	13–28	0.217

Data in Median (Q1-Q3 ranges), *: the value "28" indicates a median absence of infection between day 3 (or day 9) and day 28.

Table 3A
Whole-body protein kinetics (Leucine infusion rate = 0.1 umol/kg/min).

	Exogenous Leu (mg/kg/min)	Leucine rate of appearance (mg/kg/min)	Leucine oxidation (mg/kg/min)	Non oxidative Leu disposal (mg/kg/min)	Endogenous protein degradation (Leu Ra-Exo) (mg/kg/min)
Day 4					
EN	0.026 (0.0–0.055)	0.198 (0.142–0.229)	0.048 (0.037–0.068)	0.135 (0.087–0.186)	0.167 (0.121–0.173)
SPN	0.064 (0.053–0.078)[#]	0.209 (0.171–0.261)	0.058 (0.029–0.091)	0.145 (0.100–0.223)	0.138 (0.111–0.199)
Day 9					
EN	0.054 (0.017–0.071)	0.199 (0.164–0.225)	0.051 (0·043-0·056)	0.145 (0.117–0.171)	0.149 (0.101–0.174)
SPN	0.077 (0.058–0.094)[b]	0.277 (0.226–0.312)*	0.099 (0·069-0·161)[c]	0.176 (0.130–0.208)	0.163 (0.129–0.227)

[#]$p = 0.0022$; *: $p = 0.0071$; b: $p = 0.066$; c: $p = 0.004$; Data as median (IQR).

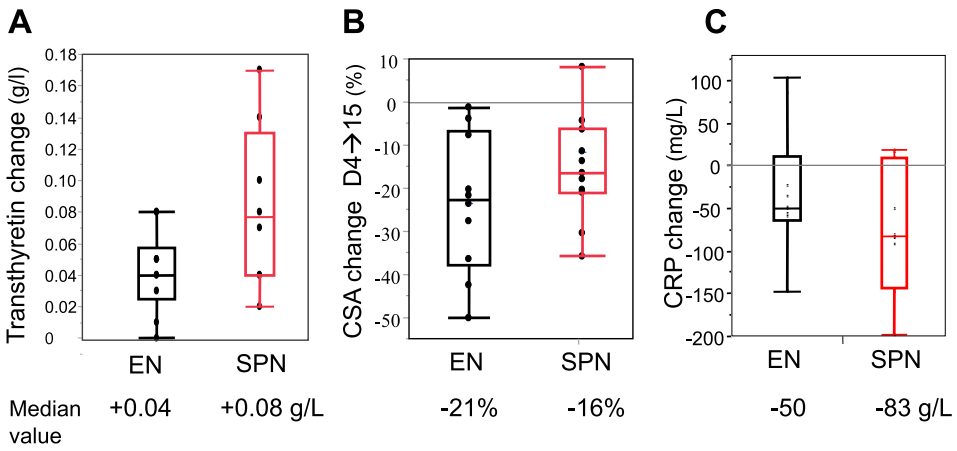

Fig. 4. A: Increase of plasma transthyretin and between D4 and D15 in both groups. The increase was significantly less in EN group: p = 0.0459. B: Decrease of muscle mass reflected by the reduction in CSA (cross sectional area of the calf) between D4, and D15 after admission (p = 0.068). C: Change of C-reactive protein (CRP) between D4 and D15 in both groups (p = 0.166).

Table 3B
Carbohydrate metabolism (Glucose infusion rate = 0.04 mg/kg/min).

	Exogenous glucose GR Exo	Glucose rate of appearance Ra (mg/kg/min)	Endogenous glucose production EGP (mg/kg/min)	Gluconeogenesis corrected* GNG (mg/kg/min)	Plasma glucose (mmol/l)	Insuline u/h
Day 4						
EN	0.685 (0.03–1.27)	2.54 (1.92–3.35)	1.85 (1.27–3.17)	0.89 (0.24–1.87)	7.5 (6.9–8.6)	1 (0–3)[c]
SPN	1.90 (1.53–2.42)[a]	3.21 (1.62–4.79)[b]	1.36 (0.52–3.53)	0.94 (0.20–3.45)	7.9 (7.1–8.9)	3.75 (2.4–5.25)
Day 9						
EN	0.94 (0.004–2.73)	2.54 (1.21–3.07)	1.28 (0.27–2.52)	0.91 (0.34–2.24)	7.1 (6.5–8.5)	0 (0–1.25)[f]
SPN	1.97 (1.40–2.83)[d]	3.04 (2.10–4.79)[e]	1.12 (0.06–3.12)	1.21 (0.01–2.11)	7.6 (6.9–8.1)	2.25 (0.75–3.6)

*: GNG was corrected for the isotopic losses into the PEPCK cycle.
[a]: p < 0.0001; [b]: 0.028; [c]; p = 0.033; [d]: p = 0.005; [e]: p = 0.017; [f]: p = 0.017 on respective days.

blood glucose within defined ranges. The increase in insulin was proportionate to the glucose dose. Endogenous glucose production, while attenuated, failed to be suppressed by nutrition at D9 in either group. Gluconeogenesis accounted to about 100% of residual glucose production. Whatever the mechanism, it was not differently affected by the nutrition strategy.

In this patient population, which reproduced the characteristics of the prior SPN-1 trial [1], the individually adapted feeding appears to affect mainly the immune function, and subsequently the rate of infectious complications. It is challenging to interpret immunological results, derived from ex vivo studies on serum, or in vitro studies on PBMC, since both compartments merely reflect the vascular compartment, and may not be fully representative of what may happen in tissues. Nonetheless, as a consequence of a decreased rate in infectious complications, in line with decreased interactions of the mainly innate immune network with bacterial by-products, or bacterial antigens, immunological studies were consistent with a lower level in proinflammatory cytokines such as IL-6, and IL-1β in the serum, or TNF-α in mitogen stimulated PBMC in the SPN group at D9. The changes in serum, or in cell secretion of these key cytokines may possibly correlate with improved antibacterial defences, and consecutive downregulation in proinflammatory cytokines usually strongly upregulated in the presence of bacterial by-products [14]. The simultaneous decrease in serum IL-10 was also supportive of the lower systemic inflammatory burden in the SPN group, and a decreased need in this key counterregulatory cytokine [15]. The enhanced proliferative response to mitogen, and the higher number of responders were also compatible with an improved immune reactivity of immune cells, in particular T cells. In contrast, the decrease in IL-8 secretion (presumably by T cells), a potent chemokine able to attract macrophages, and polymorphonuclear cells on inflammatory/infectious sites upon recall antigen stimulation of PBMC, was in line with a potential defect in innate immune response in the EN group, possibly linked to an insufficient feeding [16]. These results were in line in this same EN group with a decrease in TNF-α and IFNγ secretion, two cardinal Th1 cytokines, usually strongly induced in CD4 T cells mainly by bacterial, or viral antigens, suggesting a defective adaptive immune response also in this case.

With only 23 patients, the lower number of infectious complications observed in the SPN group is a trend. However, when these data were aggregated with those of the SPN-1 patients, the analysis shows a reinforcement of the reduction of infections (p = 0.0129), with a significantly lower probability to develop an infection after D9 with SPN (Fig. 1- Web appendix). The reduction of infectious complications is associated with a similar difference in the cumulated energy balance, as in the previous study [2], where each 1000 kcal decrease in cumulative energy deficit was associated with a 10% reduction in the risk of nosocomial infection (p < 0.05).

Ultimately, the energy balance is vital [17], but the optimal timing of reaching this equilibrium in critical illness remains debated. The patients received the value of energy expenditure (EE) measured by indirect calorimetry. The hypothesis that the measured EE corresponds to the needs remains debated, in particular regarding the optimal timing of extrinsic coverage of this value, because of the difficulty of determining the endogenous energy production, and the related risk of exceeding of needs. In the present study, the strategy to cover without exceeding the measured EE was applied from D4 (not from day 1) and was associated with a lower infectious complication rate. The cytokine changes support that this reduction is due to a modulation of immunity. Timing of feeding appears more important than the route. Indeed, the study differs from other indirect calorimetry studies in

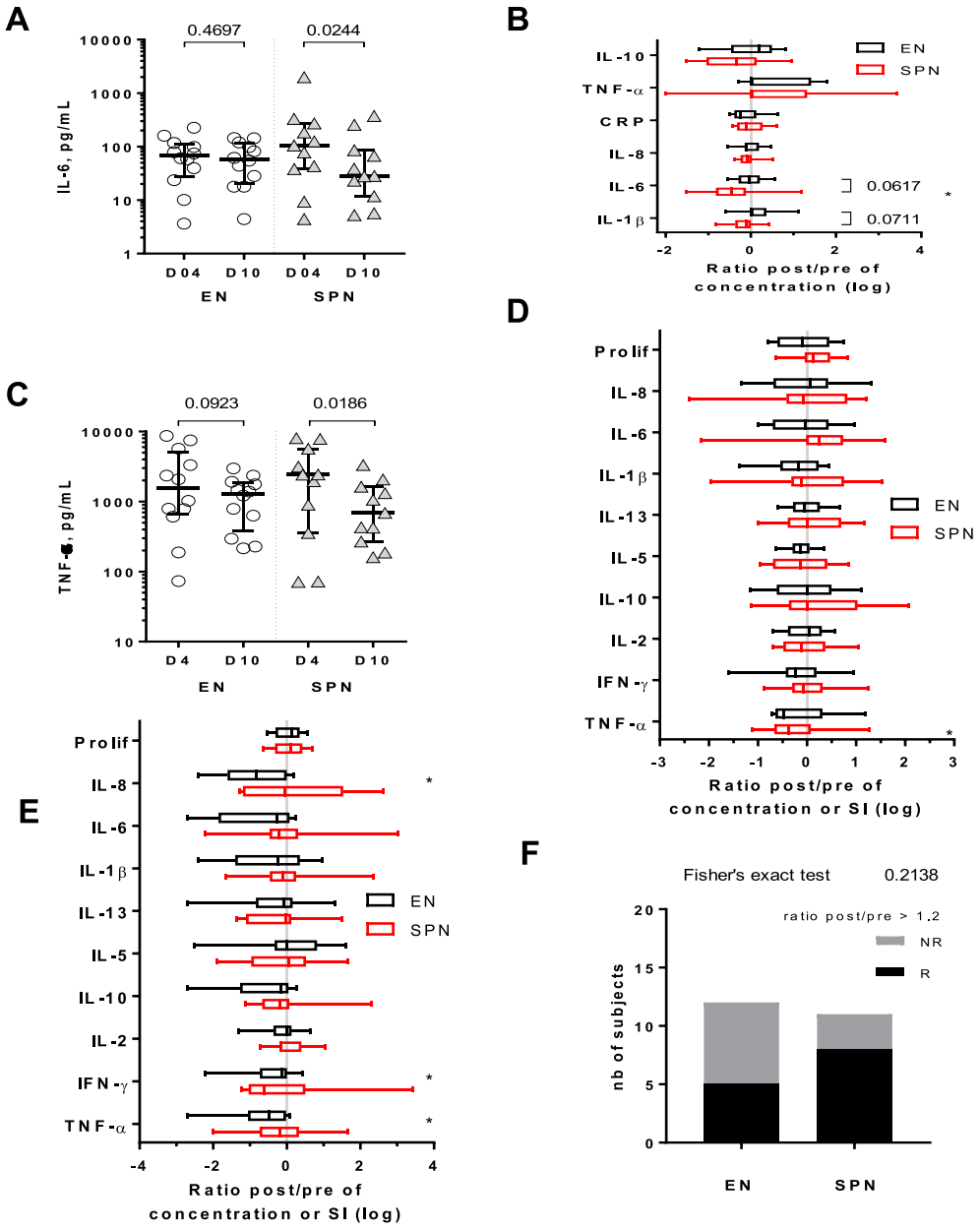

Fig. 5. Inflammatory and Immunological markers.A: Serum IL-6 at D4 and D9 in both groups; **B:** Serum IL-10, TNF-α, CRP, IL-8, IL-6 and IL-1β in EN and SPN groups, expressed as ratio post/pre of concentrations. (C to F) PBMC responses to in vitro stimulation with mitogen PHA (C, D and F) or recall antigens (E). **C:** TNF-α response of PBMC *in vitro* to PHA. **D and E:** proliferation and cytokine productions expressed as ratio post/pre of the stimulation indices or of concentrations in the 2 treatment groups. **F:** Proliferative response as numbers of responders are compared in the two groups. Responders (R) have a minimum increase of 20% of their proliferative response to PHA. Lines show median values and IQRs (A and C). Boxes show median and IQRs; wiskers are 5th and 95th percentiles (B, D and E). P values from the Wilcoxon tests (A and C), Mann.Whitney (B) and Fisher's exact test (F); *, p<0.05 Wilcoxon test of comaprison post vs. pre (B, D and E).

that the feeding to measured EE value occurred only by D4, after a modest −4000 kcal energy deficit had accumulated. The EAT-ICU trial [18] enrolled 199 mechanically ventilated patients to full nutrition from day 1: the authors showed no benefit of the EE measurement strategy, possibly because they realised early full feeding. This early strategy does not consider the energy value of the endogenous glucose production, which may reach 300 g glucose/day in young trauma patients [13], and was approx. 200 g glucose/day in the present study on D4, decreasing thereafter. Several trials have confirmed that early full feeding based on equation estimated targets is potentially deleterious. The INTACT study in ARDS patients [19] was interrupted due to four early deaths in the full feeding group, which was possibly due to a refeeding syndrome as the patients had been 7–8 days without feeding before receiving full feeding (target 30 kcal/kg). The individual target were not verified by indirect calorimetry [19]. The ANZIC-Refeeding trial compared a progressive restricted feeding to standard feeding [20], and showed mortality benefit from early limitation, and progression over 3–4 days to target.

The feeding route seems to be less important than the dose and its timing: two large randomised trials in ICU patients comparing EN and PN aiming at full early feeding, with an equation based target: 1) the CALORIES trial [21] enrolled ventilated patients showed no difference in the 30-day mortality despite more hypoglycaemic episodes and digestive complications in the enteral group; 2) the NUTRI-REA trial which enrolled patients in septic shock showed no mortality difference but again more digestive complications in the enteral group.

Study Limitations: Due to the complexity of the investigations the number of patients is low, below the planned 28 patients. This was due to the continuous pressure to transfer to other non-university ICUs, causing the loss of three patients before the 2nd tracer study could be carried out (further two patients denyed consent). This pressure resulted in intubated patients being transferred, compromising the analysis of length of mechanical ventilation, and ICU stay. The study, oriented on metabolic variables, was by design underpowered to analyse clinical outcome. In addition, to ensure optimal indirect calorimetry during the tracer study, several patients were maintained up to one day longer than required for respiratory reasons, which occurred more frequently in the SPN group. Finally, the choice of the tracer amino acid proved to be unsatisfactory, and incomplete. Leucine reflects whole body protein: phenylalanine would have better reflected the muscle compartment. Moreover, we only used one single amino acid. It has since been shown that to measure whole-body protein turnover during ongoing EN, two distinct tracers of the same amino acid should be given [22] intravenously and enterally to correct for the splanchnic extraction. Further, to address individual organs, a more invasive approach may be required. And finally, although the control group was less fed than the SPN group, the patients were not very different by D9, which attenuated the impact of the goal directed feeding.

5. Conclusions

The present study addresses the intrinsic mechanism capable to modulate infectious complications related to optimal energy provision as defined by indirect calorimetry measurements. It does not solve the debate about energy and protein requirements [23], but it shows that covering measured EE from day 4 on, as recommended by the new ESPEN guidelines [24], is safe and potentially beneficial. Energy needs, and the timing of ramping up to measured EE values, remain a challenge. The SPN strategy which covers target from day for has a clear positive impact: it seems to provide a good compromise, avoiding both the early overfeeding risk, and the building up of energy deficit. The individualised feeding strategy was applied in patients with a moderate cumulated energy deficit during the first three days, to a target measured by indirect calorimetry resulted in both biological, and clinical advantages. While not altering protein, nor glucose handling, the SPN intervention was associated with a faster attenuation of innate inflammation, and a better response of the polymorphonuclear cells to stimulation, a reduction of infectious complications.

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Conflict of interest

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CRediT authorship contribution statement

Mette M. Berger: Data curation, Writing - original draft. **Olivier Pantet:** Data curation, Writing - original draft. **Nathalie Jacquelin-Ravel:** Data curation, Writing - original draft. **Mélanie Charrière:** Data curation. **Sabine Schmidt:** Data curation, Writing - original draft. **Fabio Becce:** Data curation, Writing - original draft. **Régine Audran:** Data curation. **François Spertini:** Writing - original draft. **Luc Tappy:** Writing - original draft. **Claude Pichard:** Writing - original draft.

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