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Continuous enteral and parenteral feeding each reduce heart rate variability but differentially influence monocyte gene expression in humans

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INTRODUCTION

During critical illness, hypermetabolism and decreased nutritional intake lead to malnutrition within days, which can complicate recovery. As an adjunct to care, specialized nutritional support, delivered as enteral or parenteral feedings, provides macro- and micronutrients until oral dietary intake can resume. By helping in part to offset the hypermetabolism of critical illness, early nutritional delivery is known to improve overall outcome and has become an essential component of modern critical care. While parenteral (intravenous) nutrition (PN) held considerable promise during its development, comparisons between parenteral and enteral feedings generally demonstrate fewer infectious complications and better clinical outcomes with enteral nutritional (EN) delivery. Therefore when no barriers to gut feeding exist, enteral feedings have emerged as the standard of care. (1–3)

Pre-clinical studies of different nutrition modalities suggest that EN helps to sustain both innate and adaptive immune function more effectively than PN.(4–7) Compared to enterally fed animals, those that received parenteral nutrition demonstrated increased gut bacterial translocation(8) and had increased mortality when challenged with bacterial lipopolysaccharide (endotoxin) or with intraabdominal infection.(9) Dysregulation of both innate and adaptive immune functions are believed to influence these adverse outcomes.

Most clinical analyses of the influence of feeding modality on immune function document increased levels of pro-inflammatory cytokines in post-operative patients that had received pre-operative PN versus those fed enterally.(10,11) Similarly, studies in healthy volunteer subjects suggest that the route of nutrition influences innate immune responsiveness. We have previously shown that a seven day period of continuous PN in healthy subjects resulted in an enhanced systemic inflammatory and acute phase response after subsequent *in vivo*

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challenge with endotoxin compared to those receiving intermittent enteral feedings. (12) Interestingly, however, a later study by Santos and others in 1994 failed to reveal differences in systemic inflammatory mediators after endotoxin-challenge between normal subjects fed orally compared to those who received seven days of PN.(13)

Proposed mechanisms underlying the relationship between innate immunity and route of nutritional delivery, enteral versus parenteral, include the alterations in the balance of gut-mediated autonomic nervous system outflows. In particular, efferent vagus nerve activity appears to highly influence peripheral immune cell populations. This “cholinergic anti-inflammatory pathway” (14–16) involves the nutrient-mediated release of cholecystokinin (CCK), which acts both centrally and locally via vagal afferent pathways to stimulate α -7-nicotinic acetylcholine receptors on immune cells, especially macrophages, by way of vagal efferents.

Although the influence of specialized nutrition support upon this pathway has not, to the best of our knowledge, been examined in *humans* subjected to differing routes of feeding, pre-clinical studies suggest that intestinal luminal nutrients influence autonomic activity, in part, by modulating vagal afferent signals and the balance of efferent autonomic signals. Intestinal gavage with long chain lipids enhances afferent vagus nerve activity via stimulation of intestinal cholecystokinin receptors.(17,18) This nutrient specific stimulation of parasympathetic activity is also associated with improved survival in a model of acute hemorrhagic stress. (19)

These observations of parasympathetic stimulation by enteral feedings led us to conduct a preliminary study with the hypothesis that differing routes of nutrient delivery, enteral versus parenteral, might have a systemic impact on autonomic activity and on the pattern of gene activation within peripheral immune cell populations. To determine if an autonomic impact was detectible in healthy subjects we compared measures of heart rate variability (HRV) to determine if continuous EN or PN differentially influenced outputs, with orally fed subjects as controls. HRV analysis is a non-invasive technique of studying patterns between successive QRS complexes using continuous EKG over various time and frequency scales. It is used to detect physiologic complexity and can reflect homeostatic feedback between organ systems such as the central nervous system and the heart. Specifically, vagal and parasympathetic tone, and sympathovagal balance can be evaluated with this technique.(20) To assess the impact of differential feeding on patterns of peripheral blood monocyte (PBM) gene expression, microarray analysis was utilized to compare continuous EN and PN groups.

MATERIALS AND METHODS

Ten healthy subjects between the ages of 18 and 36 years of age were recruited by public advertisement for participation. All subjects provided written, informed consent under guidelines approved by the Institutional Review Board (IRB) of the UMDNJ-Robert Wood Johnson Medical School. Inclusion criteria were: adults aged 18–40 years and “normal general health” as demonstrated by medical history and physical examination and by laboratory testing. Women of child bearing potential were screened for pregnancy risk and were included if utilizing reliable contraception.

Subjects underwent an initial screening visit that included the comprehensive history, physical and laboratory testing to establish suitability for inclusion; eligible subjects returned within 3 weeks for admission to the clinical research center (CRC). Throughout admission, all subjects were attended by nursing staff on a 24-hour basis. Subjects were precluded from any physical activity other than walking about the study unit.

Subjects were randomized to two feeding groups: (1) a continuous feeding parenteral nutrition (n= 7) (2) a continuous naso-gastric, enteral nutrition (**EN**) (Nutren[®] Nestle Nutrition, Minnetonka, MN) group (n=3). The feeding regimens were designed to provide a non-protein calories intake of 22–24 kcal/kg-day over each 24-hour study day. This rate of substrate administration was chosen to approximate the resting energy expenditure of unstressed normal subjects and to eliminate any confounding influence of significant under- or over-nutrition. An additional cohort (n=5) of healthy subjects, recruited screened and monitored in the same fashion were admitted to the CRC and allowed an oral diet *ad libitum* and served as the control group (**PO**) for HRV analysis.

All subjects fasted overnight beginning at 2300 hours on the day of CRC admission. Subjects in the PN group had a peripherally inserted central catheter placed by the interventional radiology department between 7:00 AM and 10:00 AM the morning following admission (Day 1). The EN feeding subjects had placement of an 8 French Dobhoff[™] silastic tube (Kendall, Mansfield MA) during the same time frame. Proper placement of all feeding devices was confirmed by radiograph. The PO group required no feeding device placement.

Whole blood was collected in EDTA and heparin tubes from patients on Day 1 prior to initiation of the PN or EN feedings. Blood was again collected after 72 hours of continuous feeding (Day 4) while the assigned feeding regimen was being administered.

Monocyte Isolation from Peripheral Blood

Blood was collected in CPT tubes (BD Biosciences) and 400 μ l of RosetteSep[®] (Stem Cell Technologies, Vancouver, BC, Canada) immediately added as previously described.(21) After 20 min at room temperature (RT), tubes were centrifuged at $1200 \times g$ for 25 min at RT. The interfacial layer was collected; the top of the CPT tube was washed and added to the interfacial layer. The monocytes were recovered by centrifugation. Residual RBCs were lysed with EL buffer (Qiagen, Valencia, CA) following centrifugation and the monocyte pellet was subsequently lysed in TRIzol[™] (Invitrogen, Carlsbad, CA) and immediately frozen at -70°C .

Assessment of monocyte purity was performed on cells triple-stained with CD66b-fluorescein isothiocyanate (FITC) (Beckman-Coulter, Miami, FL), CD2-phycoerythrin (PE) (Becton-Dickinson Biosciences, San Jose, CA) and CD33-peridinin-chlorophyll-protein complex, cyanin dye 5.5 PerCP-Cy5.5 (BD Biosciences) for 30 min at 4°C . After washing 1X with PBS containing 0.5% BSA, cells were analyzed with flow cytometry FACSCalibur[™] (Becton-Dickinson Biosciences). Data were collected and analyzed using the CELLQuest[™] software. Monocyte purity was $82 \pm 1.7\%$ (mean $\pm$ SEM) for all samples.

Preparation of RNA, cDNA, and labeled cRNA

Total RNA—Cell lysates in TRIzol were thawed and treated with chloroform. RNA was isolated from the aqueous phase and precipitated with isopropyl alcohol. Following alcohol wash, the RNA pellet was dried and dissolved in DEPC water. The quality and quantity of the isolated RNA was evaluated using the 2100 Bioanalyzer[™] (Agilent Technologies, Palo Alto, CA).

cDNA synthesis—First strand cDNA synthesis was performed using reverse transcription (SuperScriptII, Invitrogen, Carlsbad, CA) with 5 μ g of total RNA, T7-oligo (dt)₂₄ primer, DTT, and dNTP mix. Second strand cDNA synthesis was then carried out with DNA polymerase I, DNA ligase, and dNTP mix, followed by additional reaction with T4 DNA

polymerase (Invitrogen, Carlsbad, CA). Double-stranded cDNA was purified using the Focus GeneChip Sample Cleanup Module (Affymetrix, Santa Clara, CA).

cRNA synthesis—Biotinylated cRNA was synthesized from the double-stranded cDNA using GeneChip expression 3'-amplification reagents for IVT labeling (Affymetrix). using MEGAscript T7 polymerase in the presence the four natural ribonucleotides and one biotin-conjugated analog. The generated biotinylated cRNA was purified using the Focus GeneChip Sample Cleanup Module (Affymetrix).

Microarray Analysis

Steps outlined in this section were performed by the microarray core facility at this institution. Following fragmentation of the biotinylated cRNA, 15 µg was placed in hybridization cocktail, heated to 95° C, centrifuged and then hybridized to the Focus GeneChip® microarray (Affymetrix, Santa Clara, CA) for 16 hours at 45° C. Chips were then washed, stained with streptavidin phycoerythrin and scanned on the Agilent Gene Array Scanner™ (Agilent Technologies, Santa Clara, CA).

Analysis of microarray data

Focus chip data CEL files were imported, grouped and analyzed using GeneSpring™ software (Agilent Technologies, Santa Clara, CA). Primary analysis was carried out by log2 transformation followed by transformation to the median and RMA (quantile) normalization. Advanced significance analysis was performed on normalized-transformed data utilizing paired or unpaired Student's t-tests as appropriate. Unless otherwise indicated below, we further defined significant probes as those with a p value < 0.05 and 1.5-fold change (FC) from the pre-feeding baseline (Day 1) or in comparison to the alternative feeding regimen on Day 4. Data were also exported for analysis by Ingenuity Pathway Analysis (IPA)™ (Ingenuity, Palo Alto, CA) as previously described.(22)In addition, monocyte gene expression for each group was subjected to analysis by filtering for a consensus group of 266 innate immunity genes shown to be responsive to *in vivo* endotoxin stimulation.(22) The microarray data have been submitted to Gene Expression Omnibus (accession number GSE21534).

Assessment of heart rate variability

A supine recording of electrocardiographic output was obtained prior to initiation of feedings on Day 1 and at 12-hour intervals (0900 and 2100 hours) throughout the study period in all subjects. Each recording interval consisted of two consecutive 5-minute epochs. During these determinations, heart rate was monitored using a continuous electrocardiography (EKG) technique with three standard limb leads and CardioPro® 2.0 software with one Infiniti and one Procomp Plus® recorder (Thought Technology, Ltd., Montreal, P.Q., Canada). HRV parameters as well as inter-beat intervals were collected using EKG data at a rate of 256 samples/second as previously described.(23,24)

Analysis of HRV Data

In a continuous EKG record, each QRS complex was detected and the “normal-to-normal” (NN) intervals (all intervals between adjacent QRS complexes resulting from sinus node depolarization) were tabulated. For each epoch, noise artifact and irregular heartbeats were manually edited by visual inspection and interpolation prior to calculation of interbeat intervals using CardioPro® software. We analyzed each epoch as previously described. (23,24) The power spectral density then was calculated using a Fast Fourier transformation algorithm. All signals were exported in standard ASCII format to Excel and EAS 9.0 for analysis and graphics as previously described.(23,24)

Time and frequency domain measures—An analysis of HRV parameters for both time domain and frequency domain measures was performed for each recorded epoch. Time domain measures included, 1) the ‘standard deviation of the average beat to beat intervals’ (SDANN), which measures total heart rate variability and overall system adaptability, 2) the ‘square root of mean squared successive differences’ (RMSSD) of interbeat intervals, considered to reflect mainly vagus nerve pathways and 3) the ‘percentage of successive interbeat interval differences greater than 50 ms’ (pNN50), associated with respiratory sinus arrhythmia and, therefore, also vagus nerve activity. Frequency domain measures included, 1) high frequency variability (HF)[0.15–0.4Hz] which correlates with parasympathetic and vagal tone, 2) low frequency variability (LF)[0.04–0.15 Hz] and 3) the low frequency/high frequency ratio (LF/HF) utilized as a measure of sympathetic/parasympathetic balance.(25)

Statistical analysis of HRV parameters

Feeding route differences in parameters of HRV were determined by two-way, repeated measures analysis of variance using Statistica® version 6.1 (StatSoft, Inc., Tulsa, OK). P-values less than 0.05 were considered to be statistically significant. Changes due to diurnal variation were not considered in this pilot study

RESULTS

Study subjects

Characteristics of the subjects completing the study and the nutritional content of these dietary formulations are shown in Table 1. There were no changes in any parameter of biochemical screening during the course of feeding and all blood glucose levels remained < 120 mg/dl (data not shown).

Measures of Heart Rate Variability

Time domain parameters of HRV—The results for time domain parameters are shown in Figure 1 as the change ($\bar{x} \pm \text{SEM}$) from baseline level for the duration of the feeding period. The time domain measures of HRV include one of overall autonomic adaptability (1A, SDANN), as well as measures reflecting predominantly parasympathetic activity (1B, pNN50; 1C, RMSSD). During the 72-hour observation period, subjects in the oral diet (PO) group experienced neither decreased autonomic adaptability nor diminished parasympathetic function as reflected by the HRV data. In contrast, both the EN and PN groups exhibited a significant decrease ($p < 0.001$) from baseline in all three time domain parameters (SDANN, pNN50, and RMSSD) over the feeding period. In addition, comparisons *between* groups (PO, EN and PN) both of overall variability and of variability over time were made. Comparing PO versus EN group revealed significant decreases in SDANN and pNN50, between the groups (main effect; $p < 0.05$) and over time (interaction effect; $p < 0.05$), after 72 hours of EN. When the PO and PN groups were compared, all time domain parameters, SDANN, pNN50, and RMSSD, were significantly diminished (main effect; $p < 0.02$), as were changes over time (interaction effect; $p < 0.02$) after 72 hours of PN. Between EN and PN groups, there were no significant differences in time domain parameters or in changes over time. These HRV findings demonstrate that, compared to oral feeding, *both* continuous EN and PN lead to diminished autonomic adaptability and decreased parasympathetic function which worsens over time as nutrition is delivered.

Frequency domain parameters of HRV—The results for frequency domain parameters for each feeding group are presented in Figure 2 as the change from baseline ($\bar{x} \pm \text{SEM}$). Frequency domain parameters reflect HRV *waveform analysis*; commonly reported measurements include low frequency (LF)[0.04–0.15 Hz], high frequency (HF)[0.15–0.4Hz] and their ratio (LF/HF). In general, HF power measurements reflect vagal tone and

parasympathetic activity; LF measures combined sympathetic and parasympathetic signaling; LF/HF ratios reflect overall sympathovagal balance. Similar to time domain measurements, subjects in the PO group experienced no differences in any of the frequency domain parameters during the 72-hour observation period. However both continuous EN and PN groups each experienced a decline from baseline in both HF power ($p<0.001$) and in LF power after 72 hours of continuous artificial feeding (2B, LF) ($p<0.05$). The ratio of LF/HF remained unchanged in all groups in comparison to baseline and over time (2C.) In comparing the EN to the PO group, HF power decreased significantly both overall (main effect; $p<0.05$) and over time (interaction effect; $p<0.05$). Comparisons of PN and PO groups demonstrated decreased HF power overall (main effect; $p<0.02$) and over time (interaction effect; $p<0.001$). No differences were seen in LF. There were no significant differences *between* EN and PN for any frequency domain measures. The diminished HF power identified in the EN and PN groups indicates a significant decrease in vagal tone and in parasympathetic activity after 72 hours of these continuous feeding modalities.

Peripheral blood monocyte gene expression

The number of differentially expressed gene probes detected in PBM after 72 hours of parenteral or enteral feeding *compared to baseline* expression prior to nutritional delivery is summarized in Table 2. For each group, differentially expressed probes are shown at the level of initial significance ($p<0.05$) and for more highly expressed differences (both $p<0.05$ and 1.5 fold-change). The complete listing of these gene probes is provided as on-line Supplemental Tables 1 and 2. Very few gene expression changes met significance and fold-change criteria after 72 hours of EN. After 72 hours of PN, 471 genes had significant changes with 73 also meeting fold-change criteria.

Between groups, the number of differentially expressed probes detected in PBM after 72 hours of PN as compared to expression after 72 hours of EN is shown in Table 3. A detailed listing of these gene probes is provided as on-line Supplemental Table 3. There were 854 significant differentially expressed genes between EN and PN groups with 157 changes meeting fold-change criteria.

Innate immune specific gene expression

Filtering expressed genes for consensus innate immunity probes identified a number of significantly expressed ($p<0.05$) genes over 72 hours of PN. These differentially expressed innate immunity genes in PBM are shown in Table 4. A small number of genes met the criteria for significance and 1.5 fold change, including up-regulated genes for STAT3, TLR4, STAT2, and down regulated genes for TRAF5 and KLHD2.

DISCUSSION

Enteral and parenteral feeding are crucial adjuncts to care during critical illness and both have been studied extensively in the ICU population. To our knowledge this pilot study is the first, however, to assess the influence of common nutritional support modalities on parameters of autonomic activity in healthy human subjects and to correlate them with immune cell gene expression in that population. The present study documents that even relatively brief periods of continuous nutrition, either enteral or parenteral, alter measures of heart rate variability with a reduction in host adaptability and overall parasympathetic outflow. Furthermore, because the subjects in the study were healthy, normal subjects, the data suggest that the use of either continuous EN or PN influence measures of autonomic activity in the absence of disease co-morbidities or other inflammatory conditions. Changes in autonomic activity, similar to those observed in the present study, have also been noted in stressed patients and have correlated to increased morbidity and mortality.(26–29)Hence, the

current observations may be important to the influence of nutritional support technologies on organ systems that depend on rhythmic autonomic signals for optimal function,(30,31) as well as on the use of these technologies in the critically ill population.

In addition to the influence of altered autonomic activity on organ system function, there is considerable evidence to suggest that the present findings, specifically that continuous EN, not just PN, depresses parasympathetic activity, may negatively impact autonomic regulation of innate immunity. Animal experiments suggest that much of the vagally-mediated influence over TNF α production occurs within tissue-fixed monocyte/macrophage cell populations.(32) In the present study we observed that the *in vivo* reduction in HRV parameters reflecting parasympathetic/vagus nervous activity (RMSSD, pNN50, HF) during continuous PN or EN did not influence *ex vivo* whole blood immune cell responsiveness to endotoxin (data not shown). This observation is consistent with evidence that vagal signaling has a prominent influence, not so much on circulating immunocytes, but rather on splenic or other splanchnic tissue sites of TNF production and on enhanced splanchnic TNF production during PN in humans. (12)

How these pathways are influenced by feeding schedule, continuous versus intermittent, is unknown. We postulate that the well-described “cholinergic anti-inflammatory pathway,” mediated by the vagus nerve, is operative involving CCK stimulation of α -7-nicotinic acetylcholine receptors on immune cells.(15,16) In an interesting series of studies, intermittent bolus feeding, but not continuous feeding created feelings of satiety and caused appetite suppression in healthy volunteers.(33,34) These and other studies(35,36) demonstrate that the induced neuro-endocrine environment may be different between the two feeding modalities. A number of mechanisms, alone or in concert may be involved. Continuous feeding may lead to tonic stimulation of neuro-endocrine pathways and deplete preformed/stored hormones or may cause changes in neurotransmitter receptor numbers or activation thresholds. Alternatively, the lower luminal concentrations of nutrients, as present in continuous feedings compared to large bolus feedings, may not reach threshold levels necessary to stimulate gut hormone release. Also, gastric distention, which is known to stimulate vagal afferents,(37)and as experienced with normal oral intake or during intermittent bolus enteral feeding but not during continuous enteral feeding, may be contributory.(34) Whatever the mechanism, our preliminary findings using HRV analysis to assess vagal tone, clearly demonstrate diminished parasympathetic output over time with continuous feedings, both enteral and parenteral.

Despite nearly identical patterns of altered autonomic activity between EN and PN subjects, differing patterns of peripheral blood monocyte (PBM) gene expression were observed between groups. Continuous EN, utilizing normal gastrointestinal nutrient absorption, differentially influenced the expression of only a few genes when compared to the pre-feeding samples. However, for those subjects who received continuous PN, a direct infusion of nutrients into the systemic circulation that bypasses the gut and normal hepatic metabolism, many genes (n=471) were differentially expressed compared to pre-feeding samples.

This study is the first to use PBM gene expression profiles as a means to compare enteral and parenteral feeding and the differences between them are profound. Distinct patterns of circulating mononuclear cell gene expression have also been identified acutely in response to individual oral dietary constituents(38) as well as more chronic interventions with potentially immuno-modulatory diets.(39) Interestingly, the limited change in PBM gene expression after 72 hours of continuous EN suggests a rapid adaptation, from a metabolic perspective, to this continuous feeding modality in healthy subjects. The findings of divergent PBM gene expression contrast sharply with the nearly identical alterations noted

in HRV/autonomic outflow, between the EN and PN groups, over the study period and suggest that very different processes are responsible for such disparate results.

Previous animal studies document tissue-specific changes in local innate immune gene expression based on route of feeding - including upregulation of Toll-like receptors (TLRs) (40) which are critical to normal immune surveillance and tolerance.(41,42)That our parenterally fed subjects also demonstrated up-regulation of monocyte TLR-4 transcripts suggests that PN influences expression of pattern recognition receptors (PRRs). This finding along with those from a previous study by our group in humans demonstrating increased monocyte cell surface TNF receptor expression after PN(43)suggests that PN promotes a state of enhanced immune cell surveillance for some PRR and damage associate molecular pattern(DAMP) ligands.

Specialized nutritional support, as delivered in the critical care setting, imposes systemic and tissue substrate fluxes and nutrient entrainment cues very different from those experienced with normal oral feeding.(44–46) These unnatural nutrient signaling patterns, leading to altered autonomic signaling as documented by the HRV findings of the present study, may represent an additional mechanism of nutrition support risk.(47,48) Based on these findings and others, we believe it is likely that both the route (enteral versus parenteral) and *timing* (intermittent versus continuous) of substrate administration influence organ system functions in complex clinical scenarios.(11–13,49,50) During this study, the profound alterations in monocyte gene expression after parenteral nutrition, compared to enteral, do not appear to be related to the changes in HRV/vagal activity and likely represent separate consequences of the direct delivery of intravenous nutrition.

The present preliminary study has identified a heretofore-undocumented influence of specialized nutritional support modalities on the regulation of heart rate variability and on autonomic activity. As investigators continue to explore the influence of vagal tone on limiting destructive hyperinflammation,(14,15) the depression of parasympathetic tone during continuous enteral feeding may be shown to contribute to overall inflammatory risk different from those associated with PN. Nutrient delivery regimes that consider both the route *and timing* of delivery, and therefore long-evolved circadian, neuro-humoral, and inflammatory mechanisms, may be more physiologic and should more widely studied in critically ill patients requiring specialized nutritional support.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

HRV *time domain* measures comparing subjects receiving continuous enteral nutrition (EN), continuous parenteral nutrition (PN), and standard oral feeding (PO) over 72 hours. (A) Standard deviation of average beat-to-beat intervals (SDANN); (B) Percentage of interval differences of successive interbeat intervals greater than 50 ms (pNN50) and (C) Square root of mean squared differences of successive interbeat intervals (RMSSD). Data points depict the Mean $\pm$ SEM. For each time domain measure, differences *within* each group over time, as well as *between* groups, both overall and over time, were assessed by 2-way analysis of variance with repeated measures on time. Both EN and PN groups showed significant differences over time for all three measures ($p < 0.001$) while the PO group had no difference over time. Compared to the PO group, EN subjects experienced decreased SDANN and pNN50 both overall and over time ($p < 0.05$); PN subjects experienced decreases in all measurements, SDANN, pNN50, and RMSSD both overall and over time ($p < 0.02$) compared to PO subjects. There were no statistically significant differences between EN and PN subjects for any of the time domain measures. These findings demonstrate that continuous EN and PN lead decreased parasympathetic function, which worsens over time as nutrition is delivered.

Figure 2.

Heart rate variability (HRV) *frequency domain* measures comparing subjects receiving continuous enteral nutrition (EN), continuous parenteral nutrition (PN), and standard oral feeding (PO) over 72 hours. (A) Power in the high frequency band [0.15–0.4 Hz] (HF); (B) Power in the low frequency band [0.04–0.15 Hz] (LF) and (C) LF/HF ratio. Data points depict the Mean ± SEM. For each frequency domain measure, differences *within* each group over time, as well as *between* groups, both overall and over time, were assessed by 2-way analysis of variance with repeated measures on time. Both EN and PN groups showed significant differences over time in HF power ($p < 0.001$) and LF power ($p < 0.05$). Compared to the PO group, both the EN and PN groups demonstrated decreased HF power both overall ($p < 0.05$ and $p < 0.02$ respectively) and over time ($p < 0.05$ and $p < 0.001$) respectively. There were no statistically significant differences between EN and PN for any of the frequency domain measures nor were there differences within the PO group over time. Compared to PO subjects, LF and LF/HF measurements were not significantly different. These findings of diminished HF power the EN and PN groups indicates a significant decrease in vagal tone and in parasympathetic activity after 72 hours of these continuous feeding modalities.

Table 1

Demographics and nutritional intake of study subjects

	PN	EN (Nutren)	PO
Age ^I	26 ± 4	32 ± 3	19 ± 1
BMI ^I	25 ± 2	28 ± 2	23 ± 1
Weight (kg) ^I	71 ± 6	79 ± 11	70 ± 5
Male/Female	4/3	2/1	5/0
<u>Daily Nutritional Intake</u>			
Protein			
g/kg	1.2	1.2	
% total calories	16%	16%	
Nitrogen/24 hrs (g)	13	15	
Dextrose			
g/kg	5.4	3.7	
% total calories	67%	51%	
Fat			
g/kg	0.6	1.1	
% total calories	17%	33%	
Total calories	2207	2294	
Non protein kcals	1823	1926	

^IMean ± SEM

Table 2

Number of genes that are either up regulated or down regulated in peripheral blood monocytes after 72 hours of continuous PN or EN as compared to respective baselines

	<u>Parenteral Nutrition</u>		<u>Enteral Nutrition</u>	
	Down	Up	Down	Up
Significant change ($p < 0.05$)	279	192	50	41
Significant change ($p < 0.05$) and & Fold change 1.5	60	13	16	2

Table 3

Number of genes that are either up regulated or down regulated in peripheral blood monocytes from PN group as compared to EN group after 72 hours of continuous feeding

	Parenteral Nutrition vs <u>Enteral Nutrition</u>	
	Down	Up
Significant change ($p < 0.05$)	528	326
Significant change ($p < 0.05$) and & Fold change 1.5	79	78

Table 4

Innate immune function genes significantly up regulated and down regulated after 72 hours of continuous parenteral feeding compared to baseline

Gene symbol	Gene Title	FC ^I
STAT3	Signal transducer and activator of transcription 3	1.876
TLR4	Toll-like receptor 4	1.810
STAT2	Signal transducer and activator of transcription 2	1.509
NFE2	Nuclear factor (erythroid derived 2), 45kDa	1.449
IL6ST	Interleukin 6 signal transducer	1.327
IFNGR2	Interferon gamma receptor 2	1.244
CD27	CD27 molecule	1.222
NOD1	Nucleotide binding oligomerization domain contain	1.149
TLR3	Toll-like receptor 3	-1.093
ORL1	Oxidized low density lipoprotein (lectin-like)	-1.094
CDK4	Cycline dependent kinase 4	-1.123
RPS6KA5	Ribosomal protein S6 kinase, 90 kDa, polypeptide 5	-1.220
TBK1	TANK-binding kinase 1	-1.221
JAK1	Janus kinase 1 (aprotein tyrosine kinase)	-1.230
IL18R1	Interleukin 18 receptor 1	-1.246
ATM	Ataxia telangiectasia mutated	-1.247
SIRT1	Sirtuin (silent mating type information regulation 2)	-1.314
BM11	BM11 polycomb ring finger oncogene	-1.320
SOD1	Superoxide dismutase 1, soluble	-1.328
IRF8	Interferon regulatory factor 8	-1.343
IRAK1	Interleukin-1 receptorassociated kinase 1	-1.349
IL1R2	Interleukin 1 receptor, type I	-1.364
IL1R1	Interleukin 1 receptor, type II	-1.385
ACBD3	Acyl Co-enzyme A binding domain	-1.391
IKBKAP	Inhibitor of kappa light polypeptide gene enhancer Nuclear factor of kappa light polypeptide gene	-1.447
NFKBIA	enhancer	-1.450
CRY1	Cryptochrome 1 (photolyase-like)	-1.498
TRAF5	TNF receptor associated factor 5	-1.566
KLHDC2	Kelch domain containing 2	-1.590

^IFC = fold change