Caloric Restriction Alters the Metabolic Response to a Mixed-Meal: Results from a Randomized, Controlled Trial

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Abstract

Objectives: To determine if caloric restriction (CR) would cause changes in plasma metabolic intermediates in response to a mixed meal, suggestive of changes in the capacity to adapt fuel oxidation to fuel availability or metabolic flexibility, and to determine how any such changes relate to insulin sensitivity (S_i).

Methods: Forty-six volunteers were randomized to a weight maintenance diet (Control), 25% CR, or 12.5% CR plus 12.5% CR energy deficit from structured aerobic exercise (CR+EX), or a liquid calorie diet (890 kcal/d until 15% reduction in body weight) for six months. Fasting and postprandial plasma samples were obtained at baseline, three, and six months. A targeted mass spectrometry-based platform was used to measure concentrations of individual free fatty acids (FFA), amino acids (AA), and acylcarnitines (AC). S_i was measured with an intravenous glucose tolerance test.

Results: Over three and six months, there were significantly larger differences in fasting-to-postprandial (FPP) concentrations of medium and long chain AC (byproducts of FA oxidation) in the CR relative to Control and a tendency for the same in CR+EX (CR-3 month P = 0.02; CR-6 month P = 0.002; CR+EX-3 month P = 0.09; CR+EX-6 month P = 0.08). After three months of CR, there was a trend towards a larger difference in FPP FFA concentrations (P = 0.07; CR-3 month P = 0.08; CR+EX-3 month P = 0.08). Time-varying differences in FPP concentrations of AC and AA were independently related to time-varying S_i (P<0.05 for both).

Conclusions: Based on changes in intermediates of FA oxidation following a food challenge, CR imparted improvements in metabolic flexibility that correlated with improvements in S_i.

Trial Registration: ClinicalTrials.gov NCT00099151

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Introduction

Caloric restriction provides metabolic benefits in a variety of nonhuman animal species (reviewed in [1]). There is great interest in determining whether these metabolic benefits translate to humans and whether caloric restriction induces favorable changes in metabolic indicators of enhanced longevity. The NIH-sponsored Comprehensive Assessment of the Long Term Effects of Reducing Intake of Energy (CALERIE) was a Phase I pilot study that examined the potential health benefits of caloric restriction in sedentary, non-obese, healthy individuals. While the primary aim of the original study was to determine the impact of caloric restriction on biomarkers of longevity and metabolic adaptation, the secondary aims of CALERIE were to evaluate the impact of caloric restriction on risk factors for type 2 diabetes mellitus and cardiovascular disease. In this analysis of data from the Pennington-site CALERIE, we sought to understand if caloric restriction could improve metabolic flexibility.

Metabolic flexibility is an emerging indicator of (metabolic) health [2,3]. In its most general sense, metabolic flexibility refers to the efficient variation of energy substrate utilization depending on substrate availability and energy demand, or the capacity to adapt...
fuel oxidation to fuel availability [3], and this is how we interpret the
term in this report. To preserve available glucose for use by the
brain in the fasting state, most body organs oxidize free fatty acids
and amino acids, released through lipolysis and proteolysis,
respectively. After a balanced mixed meal, glucose is oxidized in
the brain and other organs while lipolysis in adipose tissue and
glycogenolysis in the liver are inhibited by increases in insulin levels
induced by the ingested calories. Excess glucose is stored as glycogen
in the liver and skeletal muscle, and ingested lipids are stored as fat,
preferentially in adipose tissue. Efficient substrate switching after a
mixed meal manifests as a decrease in fatty acid oxidation and a fall
in circulating free fatty acids and intermediates of fatty acid
oxidation [2,3]. Impairment in this normal mode of substrate
switching is associated with obesity, skeletal muscle insulin
resistance, metabolic syndrome, and type 2 diabetes mellitus [3,4,5].

Prior studies have examined the effects of type 2 diabetes, obesity,
insulin resistance, and a family history of type 2 diabetes on
metabolic flexibility, defined as the ability to shift substrate oxidation
and measured by whole body and skeletal muscle respiratory
quotient (RQ) [3]. Such investigations have evaluated RQ changes
in response to a hyperinsulinemic clamp, high carbohydrate or high
fat meals, and high carbohydrate or high fat diets [3].

Thus far in human studies, metabolic flexibility has been
measured as alterations in RQ, which serves as a surrogate for
substrate oxidation and ranges from 1 for total carbohydrate
oxidation to 0.7 for total fat oxidation [3]. Using changes in RQ as
the primary variable, weight loss (with and without exercise
training) has been associated with a trend towards increased
metabolic flexibility, measured in the fasting state alone or in
response to a hyperinsulinemic clamp, high carbohydrate or high
fat meals, and high carbohydrate or high fat diets [3].

As previously reported [12], a frequently sampled intravenous
infusion of eight free fatty acids (FFA), 15 amino acids (AA), and 45
acylcarnitines (AC) as described previously [11]. In brief, after
methylation with iodomethane and partial purification, FFA
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...enzyme activities over the intervention period when compared to non-caloric restricted controls.

**Methods**

**Design and Participants**

Forty-six healthy, non-smoking, overweight (25≤BMI<30), 25–
50 year old men and 25–45 year old women were recruited to
participate in and completed a six-month intervention (clinical trials
number NCT00427193). The protocol for this trial and supporting
CONSORT checklist are available as supporting information; see
Checklist S1 and Protocol S1. Participants were excluded if they had
a history of cardiovascular disease, elevated blood pressure (>140/
90 mmHg), high fasting blood glucose (>126 mg/dL), chronic
medications (except oral contraceptives), smoking, regular exercise
(more than twice a week), abnormal thyroid function or abnormal
ECG. Participant flow in the trial is shown in Figure 1.

**Ethics Statement**

The study was approved by the Pennington Biomedical
Research Center (PBRC, Baton Rouge, LA) Institutional Review
Board and the CALERIE Data Safety Monitoring Board, and
written informed consent was obtained for all participants.

**Interventions**

Participants were randomized into one of four groups for 24
weeks: healthy weight maintenance diet (Control); caloric
restriction (CR): 25% caloric restriction from baseline energy
requirements; caloric restriction and exercise (CR+EX): 12.5%
caloric restriction and 12.5% increase in energy expenditure
through structured aerobic exercise; or, liquid calorie diet (LCD;
890 kcal/day) to rapidly achieve 15% weight loss followed by
weight stabilization with a eucaloric diet designed to maintain
body mass at this level. The group assignment was stratified to
ensure equal distributions of sex and BMI in the four groups, and
participants were analyzed as intention-to-treat.

**Diets**

As previously described, total energy expenditure measured
using doubly labeled water was used for calculation of energy
requirements [10]. All diets were based on American Heart
Association (AHA) recommendations (30% calories from fat, 15%
from protein and 55% from carbohydrate).

**Structured Exercise**

Individual exercise prescriptions were based on increasing
energy expenditure 12.5% above resting energy expenditure as
previously described [10]. Exercise modes included walking,
running, and cycling at a frequency of five days per week, with
at least three supervised sessions per week. Adherence was assessed
with heart rate monitors (Polar S-610).

**Metabolic Profiling**

Plasma samples were obtained after an overnight fast and 60
and 90 minutes after a standard lunch mixed meal (postprandial
and pooled) on an inpatient research unit at each of three time
points: baseline (before initiation of the intervention), three months
following initiation of the intervention and six months following
initiation of the intervention (study exit). Samples were prepared
and stored at −80°C for later analysis.

Targeted mass spectrometry was used to measure concentra-
tions of eight free fatty acids (FFA), 15 amino acids (AA), and 45
acylcarnitines (AC) as described previously [11]. In brief, after
methylation with iodomethane and partial purification, FFA
concentrations were measured with gas chromatography/mass
spectrometry (GC/MS). After methanol precipitation and
esterification, AC and AA concentrations were measured with tandem
mass spectrometry (MS/MS).

**Insulin sensitivity (SI)**

As previously reported [12], a frequently sampled intravenous
glucose tolerance test (IVGTT) was performed with 300 mg/kg of
glucose injected at baseline and 0.03 units/kg of Humulin (insulin) injected at minute 20. Concentrations of insulin (DPC 2000, Diagnostic Product Corporation, Los Angeles, CA) and glucose (Synchron CX7, Beckman-Coulter, Brea, CA) were measured at each sampling time. Using these concentrations, SI was derived using Bergman’s Minimal Model \[13,14\].

Data Analysis

Principal components analysis (PCA) was used as a data reduction technique. PCA is commonly used to reduce a large number of observed variables to a smaller number of constructed variables (principal components) that account for the variance in the observed variables \[15\]. It has been used for data reduction in prior studies of metabolic intermediates \[11,16,17,18\].

Specifically, we created a fasting-to-postprandial change score for each metabolite at each time point as follows: Change score = postprandial – fasting concentration. Then, PCA was performed using varimax rotation on the baseline change score for each metabolite class such that one PCA change score analysis was performed for each domain of FFAs, AGs, and AAs. The number of components retained for each model was selected to balance parsimony and the total percent of variance explained. Then, component loadings from baseline change PCAs were applied to the fasting to postprandial change scores for the three- and six-month time points normalized to the baseline values, and component scores were generated for each metabolic domain at each time point. This normalization provided a common metric for estimating change in components over time.

To determine whether the intervention affected change in fasting-to-postprandial differences (FPPD) over time, we used mixed models \[16\] to regress FPPD on time, intervention group and an intervention group-by-time product term. FPPD were based on the principal components described above. For these models, the intervention group-by-time product term indicated whether changes over time in FPPD vary with intervention group (versus Control). To account for potential nonlinearity of the time effect, time was modeled as a categorical variable. Thus, the omnibus intervention group x time interaction was calculated on 6 df \([3–1 = 3] [3–1 = 2]\) time points. Additionally, we used mixed models to regress time-varying insulin sensitivity \(S_I\) on time plus each time-varying FPPD component. The coefficients for each time-varying FPPD component in these models indicated whether (averaged across all time points) the FPPD components were positively or negatively related to \(S_I\).
pseudo R² statistic was used to determine the between person variance in S₁ [19].

Results

Information on the main determinants from CALERIE is reported elsewhere. Briefly, CR and CR+EX improved two biomarkers associated with longevity, reduced core body temperature and fasting insulin concentrations as well as 24-hour energy expenditure, DNA damage, and cardiovascular risk profiles [10,22]. Also, CR and CR+EX improved weight and fat mass, pancreatic beta cell function, and intrahepatic lipid content [12,23].

Fasting to Postprandial Changes in Metabolites

Using PCA to analyze changes in the fasting-to-postprandial difference (FPPD), we identified single components for FPPD for each of the major analyte modules– FFA, AC, and AA– with eigenvalues of 6.37, 8.67, and 7.88 respectively, explaining 80%, 20% and 53% of the variance in the component (Table 1). At the initial assessment, individual FFA and AC metabolites generally decreased from fasting to the postprandial state, particularly those that loaded heavily as part of the FFA and AC components (Table 2). In contrast, individual AA concentrations increased in the transition to the postprandial state (Table 2).

In response to CR, the AC FPPD was amplified (more negative FPPD) over time relative to Control (P = 0.02 at 3 mo., P = 0.002 at 6 mo., overall P<0.001) (Figure 2B). A similar trend was observed for the CR+EX group (P = 0.09 at 3 mo., P = 0.08 at 6 mo.; Figure 2B). In response to CR, the FFA FPPD was also amplified (p = 0.08 at 3 mo., p = 0.40 at 6 mo., overall p = 0.07, Figure 2A). In response to both CR and CR+EX, the change in AA FPPD was not significant (overall p = 0.87). In response to LCD, there were no sustained responses in AC, FFA, or AA FPPD (P>0.10 for all). However, the increase in FPPD for AC and FFA in subjects exposed to CR supports the conclusion that these subjects achieved an increase in metabolic flexibility as a consequence of the intervention.

Relations among S₁ exercise, and metabolite concentrations

As previously reported [12], between baseline and six months, there was significant improvement in S₁ in the CR+EX group and a trend towards improvement in S₁ in the CR group compared to Control (P = 0.01, P = 0.08, respectively). For each of these treatment groups, we observed a broad range of changes in insulin sensitivity; specifically, despite an average improvement in insulin sensitivity for each group, the individual participant responses were varied (Figure 3A). To better understand how insulin sensitivity improvements related to changes in FPPD, irrespective of how the change in S₁ was achieved, all subjects were included in these regression analyses ignoring group assignment (Figure 3B). There were no significant relations between FPPD in FFA and time-varying S₁ at 3 months (P = 0.54) or at six months (P = 0.91). In contrast, in a model that predicted S₁, given both time-varying AC and AA components, FPPD in AC concentrations were inversely related to time-varying S₁ (P = 0.04; Figure 4A), and FPPD in AA concentrations were positively related to time-varying S₁ (P = 0.04; Figure 4B). The AA and AC FPPD scores explained 18% of the between person variance in S₁. These results indicate that averaged across all time points, higher S₁ was associated with better metabolic flexibility as represented by larger changes in AC and AA FPPD scores.

Table 1. Principal Components Analyses (PCA) for Fasting to Postprandial Changes in Metabolites.*

| Constituents | Loadings | Eigen-value | Cumulative Variance |
|--------------|----------|-------------|---------------------|
| Post-Pre Fatty Acid Component 1 | | | |
| Palmitic acid | 0.96 | 6.37 | 0.80 |
| Linoleic acid | 0.95 | | |
| Oleic acid | 0.95 | | |
| Myristic acid | 0.93 | | |
| Palmitoleic acid | 0.91 | | |
| Stearic acid | 0.87 | | |
| alpha-Linolenic acid | 0.86 | | |
| Arachidonic acid | 0.66 | | |
| Post-Pre Acylcarnitine Component 1 | | | |
| C12:1 | 0.77 | 8.67 | 0.20 |
| C16 | 0.77 | | |
| C14:1 | 0.75 | | |
| C14:2 | 0.72 | | |
| C16:2 | 0.67 | | |
| C16:1 | 0.67 | | |
| C12 | 0.69 | | |
| C18:1 | 0.66 | | |
| C6-DC | 0.62 | | |
| C10:1 | 0.61 | | |
| C10 | 0.56 | | |
| C8:1 | 0.54 | | |
| C8 | 0.53 | | |
| C10-OH/C8-DC | 0.53 | | |
| C18:2 | 0.53 | | |
| C8:1-DC | 0.51 | | |
| Post-Pre Amino Acid Component 1 | | | |
| Leucine/iso-Leucine | 0.91 | 7.88 | 0.53 |
| Phenylalanine | 0.91 | | |
| Methionine | 0.87 | | |
| Histidine | 0.83 | | |
| Valine | 0.81 | | |
| Tyrosine | 0.81 | | |
| Aspartate/Asparagine | 0.81 | | |
| Serine | 0.79 | | |
| Proline | 0.71 | | |
| Ornithine | 0.66 | | |
| Arginine | 0.65 | | |
| Glycine | 0.56 | | |
| Alanine | 0.56 | | |

*Changes were computed as postprandial metabolite concentration minus preprandial metabolite concentration. For these differences, PCA was performed separately for each metabolite class: fatty acids, acylcarnitines, and amino acids. Key metabolites within each component (i.e., metabolites with component load >=0.5) are presented. doi:10.1371/journal.pone.0028190.0001

In order to determine whether larger FPPD corresponded to changes in fasting concentrations, postprandial concentrations or both, we evaluated raw data for individual metabolites loading most heavily on each component. By evaluating raw data, the
Table 2. Median and interquartile range (IQR) of fasting and postprandial concentrations of metabolites at baseline (n = 46).*

| Metabolite Changes | Preprandial Median (IQR) | Postprandial Median (IQR) |
|--------------------|--------------------------|---------------------------|
| **Free Fatty Acids** |                          |                           |
| Myristic acid, FA-C14:0 (µM) | 6.64 (4.21) | 2.00 (1.82) |
| Palmitoleic acid FA-C16:1(ω-7) (µM) | 13.19 (11.31) | 1.41 (1.01) |
| Palmitic acid, FA-C16:0 (µM) | 110.5 (60.1) | 22.9 (16.7) |
| ω-Linolenic acid, FA-C18:3(ω-3) (µM) | 5.15 (2.40) | 1.25 (1.11) |
| Linoleic acid, FA- C18:2(ω-6) (µM) | 77.4 (53.1) | 16.5 (10.9) |
| Oleic acid, FA-C18:1(ω-9) (µM) | 165.7 (64.4) | 31.9 (26.0) |
| Stearic acid, FA-C18:0 (µM) | 42.0 (18.0) | 11.0 (7.4) |
| Arachidonic acid, FA-C20:4(ω-6) (µM) | 4.18 (1.62) | 2.41 (1.60) |
| **Acylcarnitines** |                          |                           |
| Acetil carnitine, C2 (nM) | 6941.3 (3053.4) | 4169.4 (1929.1) |
| Propionyl carnitine, C3 (nM) | 386.6 (243.2) | 471.9 (273.9) |
| Butyryl/Isobutyryl carnitine, C4/Ci4 (nM) | 136.0 (112.8) | 160.3 (64.5) |
| Trigly carnitine, C5-1 (nM) | 520.0 (21.6) | 48.1 (25.1) |
| Isovaleryl/3-Methylbutyryl/2-Methylbutyryl carnitine, C5's (nM) | 102.0 (54.8) | 110.6 (61.0) |
| 3-Hydroxy butyryl carnitine, C4OH (nM) | 21.0 (18.0) | 16.9 (19.6) |
| Hexanoyl carnitine, C6 (nM) | 0 (0) | 21.3 (69.2) |
| 3-Hydroxy-isovaleryl/Malonyl carnitine, C5OH/C3DC (nM) | 92.8 (88.0) | 104.1 (111.2) |
| Methylmalonyl/Succinyl carnitine, C4DC/C4DC (nM) | 21.8 (14.1) | 22.6 (13.7) |
| Octenoyl carnitine, C8:1 (nM) | 151.4 (76.3) | 122.8 (68.4) |
| Octanoyl carnitine, C8 (nM) | 70.1 (32.5) | 29.3 (19.1) |
| Glutaryl carnitine, C5DC (nM) | 28.9 (17.7) | 23.0 (13.7) |
| Adipoyl carnitine, C6DC (nM) | 39.4 (29.0) | 27.6 (18.9) |
| Decatrienoyl carnitine, C10:3 (nM) | 96.8 (47.7) | 71.0 (41.0) |
| Decadienoyl carnitine, C10:2 (nM) | 28.5 (11.7) | 21.1 (15.2) |
| Decanoyl carnitine, C10:1 (nM) | 140.5 (67.3) | 58.8 (29.5) |
| Decanoyl carnitine, C10 (nM) | 142.1 (72.2) | 45.4 (74.1) |
| 3-Hydroxy-decanoyl/Suberoyl carnitine, C10-OH/C8-DC (nM) | 14.1 (7.4) | 6.0 (4.0) |
| Dodecenoyl carnitine, C12:1 (nM) | 57.2 (17.3) | 26.2 (12.4) |
| Lauroyl carnitine, C12 (nM) | 38.6 (23.2) | 16.2 (10.1) |
| 3-Hydroxy-dodecanoyl/Sebacoyl carnitine, C12-OH/C10-DC (nM) | 4.1 (2.5) | 1.5 (2.5) |
| Tetradecienoyl carnitine, C14:2 (nM) | 23.1 (12.2) | 6.9 (5.1) |
| Tetradecenoyl carnitine, C14:1 (nM) | 39.4 (17.7) | 12.4 (6.0) |
| Myristoyl carnitine, C14 (nM) | 12.7 (7.4) | 8.5 (4.4) |
| 3-Hydroxy-tetradecenoyl carnitine, C14:1-OH (nM) | 8.7 (5.3) | 6.0 (5.1) |
| 3-Hydroxy-tetradecanoyl/Dodecanediyl carnitine, C14-OH/C12-DC (nM) | 5.6 (3.9) | 3.5 (3.2) |
| Palmitoyl carnitine, C16 (nM) | 60.8 (20.4) | 42.4 (15.6) |
| 3-Hydroxy-hexadecanoyl/Tetradecanediyl carnitine, C16-OH/C14-DC (nM) | 2.1 (1.9) | 2.1 (1.6) |
| Linoleyl carnitine, C18:2 (nM) | 54.1 (18.3) | 38.1 (21.8) |
| Oleyl carnitine, C18:1 (nM) | 96.7 (40.3) | 61.7 (22.1) |
| Stearoyl carnitine, C18 (nM) | 26.2 (9.0) | 23.7 (11.4) |
| 3-Hydroxy-octadecenoyl carnitine, C18:1-OH (nM) | 3.9 (1.9) | 2.3 (1.8) |
| 3-Hydroxy-octadecanoyl/Hexadecanediyl carnitine, C18-OH/C16-DC (nM) | 3.2 (2.8) | 3.0 (1.8) |
| Arachidoyl carnitine, C20 (nM) | 3.5 (2.6) | 3.2 (2.5) |
| Octadecenediyl carnitine, C18:1-DC (nM) | 4.3 (2.3) | 4.0 (2.4) |
| 3-Hydroxy-eicosanoyl/Octadecanediyl carnitine, C20-OH/C18-DC (nM) | 4.7 (3.5) | 4.1 (3.7) |
| Docosanoyl carnitine, C22 (nM) | 2.2 (1.8) | 1.9 (1.6) |
| 3-Hydroxy-cis-5-octenoyl/Hexadecanediyl carnitine, C8:1-OH/C6:1-DC (nM) | 22.1 (12.2) | 16.0 (8.0) |
| Heptanediyl carnitine, C7-DC (nM) | 0 (6.2) | 0 (4.9) |
| Octenediyl carnitine, C8:1-DC (nM) | 13.5 (8.4) | 12.1 (7.3) |
intervention-induced amplifications in FPPD were attributable to changes in the fasting concentrations, as opposed to the postprandial concentration of the metabolites; that is, increased fasting AC and decreased fasting AA over time. As seen in Figure 5, in response to caloric restriction (CR), fasting AC concentrations increased while postprandial changes were variable. For AAs, despite a reduction in the AA difference in response to CR, larger pre-postprandial differences in AAs were related to higher SIs. These observations emphasize that there were not one to one correlations between CR and improvements in SIs. However, it remained unclear for those with the largest time varying SIs whether the pre to postprandial AA differences increased over time because the fasting AA concentrations decreased or the post-prandial concentrations increased. To address this, we evaluated the mean pre and postprandial AAs for participants with the six largest and six smallest predicted SIs. As seen in Figure 6, for those with high SIs changes fasting AA concentrations decreased while for those with low SIs changes, fasting AA concentrations increased or remained unchanged. Postprandial AA concentrations decreased over time both in those with the most and least SIs improvements. Therefore, for both AAs and AA s, the larger FPPD s arose primarily from changes in fasting concentrations with increases in AC s and decreases in AAs.

Discussion

In this randomized controlled trial of three healthy lifestyle interventions (CR, CR+EX, and a healthy weight maintenance diet) and a very low calorie diet, we observed that the CR intervention significantly increased the fasting-to-postprandial difference (FPPD) in circulating acylcarnitines (AC) and free fatty acids (FFA). Moreover, we observed that increased FPPD for both AC and AA were related to greater insulin sensitivity. Thus, this study expands prior investigations of metabolic flexibility from 1) acute responses to an infusion or meal to responses to a prolonged intervention, 2) responses to glucose/insulin infusions to more clinically-relevant mixed meals, 3) assessments made by increases in RQ to changes in a broad panel of metabolic intermediates that includes both substrates and products of key energy producing pathways.

The term metabolic flexibility is used to describe the efficient transition between substrate utilization in response to changes in substrate supply or energy demand. One example is the shift from the use of fatty acids as the main energy source during fasting conditions towards glucose utilization in a fed state [3]. Three months of CR led to an increased gradient in pre- to postprandial AC concentrations, primarily due to an increase in fasting AC concentrations. Since AC are intermediates in fatty acid oxidation, increased fasting AC concentrations reflect either a block in complete fatty acid oxidation or increases in lipolysis and fatty acid oxidation. These findings were supported by the concurrent increase in the gradient of pre-postprandial FFA, primarily mediated by increased fasting FFA concentrations. Since these changes occurred in the setting of caloric restriction where energy demand exceeds supply, in the fasting state when fatty acid oxidation predominates, and these changes associate with improved health outcomes, including insulin sensitivity, we believe an increase in fatty acid oxidation is more likely. Thus, these data imply that fatty acids are more effectively mobilized and oxidized in the fasted state in subjects that have undergone CR. The mechanisms underlying these effects remain to be investigated.
To our knowledge, this is the first report of a relation between an increased FPPD for AA and greater insulin sensitivity. While in prior investigations, amplified FPPD gradients in fatty acid and glucose oxidation have been recognized as signs of improved metabolic flexibility [4,8], there has been little focus on AA metabolism in this context. Our findings here are consistent with our prior studies showing that elevated fasting concentrations of branched chain amino acids (BCAA) and related metabolites are associated with insulin resistance [11,16]. Interestingly, in the current investigation, BCAA garnered the highest loadings on the AA principal component. Here, we observed that the ability to modulate AA concentrations was associated with improved insulin sensitivity, and this modulation was due primarily to a reduction in fasting AA concentrations. Further, since dietary composition was controlled and maintained constant throughout the investigation, changes in AA concentrations are not likely related to dietary changes. These observations emphasize that the concept of metabolic flexibility should not be limited to discussions of fat and glucose metabolism but should also include AA metabolism.

Here, we showed that CR (at a level of −25% from basal energy requirements) improved the ability to shift energy substrates with feeding, and that caloric restriction plus exercise (CR+EX), with an identical relative energy deficit, imparted roughly half of the ability to shift substrate seen with CR alone. In contrast, in a study by Kelley and Goodpaster, a combination of weight loss and exercise training increased the rate of fatty acid relative to glucose oxidization in the fasting state (lower fasting glucose oxidation have been recognized as signs of improved metabolic flexibility [4,8], there has been little focus on AA metabolism in this context. Our findings here are consistent with our prior studies showing that elevated fasting concentrations of branched chain amino acids (BCAA) and related metabolites are associated with insulin resistance [11,16]. Interestingly, in the current investigation, BCAA garnered the highest loadings on the AA principal component. Here, we observed that the ability to modulate AA concentrations was associated with improved insulin sensitivity, and this modulation was due primarily to a reduction in fasting AA concentrations. Further, since dietary composition was controlled and maintained constant throughout the investigation, changes in AA concentrations are not likely related to dietary changes. These observations emphasize that the concept of metabolic flexibility should not be limited to discussions of fat and glucose metabolism but should also include AA metabolism.

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Figure 4. Correlation Between Fasting to Postprandial Component Changes and Predicted $S_i$ Change Over Time. As described in Methods, fasting and postprandial concentrations of amino acids and acylcarnitines were measured at baseline, three months, and six months, and fasting to postprandial components were generated. $S_i$ was determined from insulin and glucose concentrations measured during a frequently sampled intravenous tolerance test at each of baseline, three, and six months. Linear models were used to relate time varying concentrations of fasting to postprandial amino acid and acylcarnitine component to time varying insulin sensitivity ($S_i$). Scatter plots depict the relation between fasting to postprandial component scores and predicted $S_i$. A. Relation between Acylcarnitines (AC) Fasting to Postprandial Component Scores and Predicted $S_i$ Over Time. Since postprandial AC concentrations are larger than fasting, more negative fasting to postprandial differences represent more metabolic flexibility. B. Relation between Amino Acid (AA) Fasting to Postprandial Component Scores and Predicted $S_i$ Over Time.
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Figure 5. Preprandial and postprandial concentrations of acylcarnitines in response to caloric restriction (CR). Baseline and three month acylcarnitine concentrations are shown for both fasting (preprandial) and postprandial assessments. The six acylcarnitines that had the largest loadings on the acylcarnitine factor (see Table 1) are shown.
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respiratory quotient [RQ]) as compared to before the intervention [8]; however, in the absence of exercise training, there was little change in fasting RQ with CR alone [4]. We note that our study differs from that of Kelley and Goodpaster in terms of the “feeding” challenge (clamp versus a mixed meal), assessment of substrate oxidation with metabolic intermediates rather than RQ, targeting the transition from the fasted to the fed state in contrast to their emphasis on fasting fat oxidation alone, and differences in exercise regimens or timing of assessments after training cessation [20]. Moreover, Kelley and Goodpaster observed that increased fasting fatty acid oxidation relative to glucose oxidation was highly associated with improvements in SI [8]. While we observed a relationship between the two, we noted that SI improvements were greatest in the CR+EX group, but substrate utilization improvements were greatest in the CR group. We also note that the subjects in the CALERIE study were healthy with normal insulin sensitivity prior to the CR or CR+EX interventions, whereas those studied by Goodpaster and Kelly were insulin resistant [4,8,21]. These findings suggest that while improvements in insulin sensitivity are related to metabolic flexibility changes, there are additional components of the variability of each that are yet to be identified.

Our observations add metabolic flexibility, as measured by changes in metabolic intermediates in response to a mixed meal, to the list of improvements that occur in the setting of CR elucidated through the CALERIE study. In addition to this effect, CR or CR+EX reduced core body temperature, 24-hour energy expenditure, fasting insulin concentrations, DNA damage, and cardiovascular risk profiles [10,22]. Matched for energy deficit, CR and CR+EX produced similar improvements in reductions in weight and fat mass, pancreatic beta cell function, and intrahepatic lipid content [12,23]. In contrast, while both CR and CR+EX increased adiponectin concentrations, and skeletal muscle sirtuin 1 (SIRT1) expression, these increases were more pronounced in the CR alone group, suggesting that these effects were primarily mediated by the CR component of the combined intervention [24]. Similarly, 25% CR produced a greater metabolic adaptation (as defined by a reduction in energy expenditure) than would be predicted for loss in body mass alone, and that was not observed with 12.5% CR+EX [25].

Figure 6. Preprandial and postprandial concentrations of amino acids for those with the highest and lowest insulin sensitivity changes. Baseline and three month amino acids concentrations are shown for both fasting (preprandial) and postprandial assessments. The five amino acids that had the largest loadings on the amino acid factor (see Table 1) are shown. Leu/Ile = leucine/isoleucine, Phe = phenylalanine, Met = Methionine, His = Histidine, Val = Valine. doi:10.1371/journal.pone.0028190.g006
The careful control of the caloric restriction and exercise interventions is a great strength of this analysis. To minimize type I error rates, we used PCA to reduce the dimensionality of the data. We performed these analyses cognizant that using PCA in small samples can result in ‘overfitting,’ where findings are sample-specific rather than representative of the population of interest [26]. However, the PCA-derived components and loadings here were both substantively credible and consistent with findings from previous studies using PCA on the same or similar metabolite measures [11,16,17,18]. With a sample size of 46, we had limited power to observe small to medium sized effects, and given this, we present statistical trends (0.05<P<0.10) in several instances. Additionally, while the metabolite profiles can identify perturbations in systemic trafficking of specific carbon fuels, they do not provide definitive information about metabolic flux. Nonetheless, the results of such analyses provide a valuable guide for further investigations seeking to identify underlying mechanisms.

In summary, CR improved metabolic flexibility evidenced by higher fasting AC and FFA concentrations and widened FPPD gradients for these metabolites. Furthermore, the change in the FPPD gradient of AC and AA concentration was related to improvements in insulin sensitivity.

Supporting Information

Checklist S1  CONSORT Checklist. (DOC)

Protocol S1  Trial Protocol. (DOC)

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Author Contributions

Conceived and designed the experiments: ER LMR WEK CBN VBK. Performed the experiments: RDS MJM BRW JRB. Analyzed the data: KMH LRL CFP VBV W. Wrote the paper: KMH LMR LRL CFP RDS MJM BRW JRB VBV. Contributed reagents/materials/analysis tools: KWM BEA LRL TKG MRR VCD OFC GAH DJW KWM CKL DWJ LRH SJS WJL LBM VBM. Analyzed the data: KWM BEA LRL TKG MRR VCD GAH DJW KWM CKL DWJ LRH SJS WJL LBM VBM. Contributed reagents/materials/analysis tools: KWM BEA LRL TKG MRR VCD GAH DJW KWM CKL DWJ LRH SJS WJL LBM VBM. Wrote the paper: KWM BEA LRL TKG MRR VCD GAH DJW KWM CKL DWJ LRH SJS WJL LBM VBM.

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