Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men

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ABSTRACT

Background
In animal experiments, high plasma concentrations of non-esterified fatty acids (NEFA) are associated with increased triglyceride stores in liver and heart, and impaired cardiac function. In humans, caloric restriction increases plasma NEFA levels. The purpose of this study was to assess the effects of progressive caloric restriction on myocardial and hepatic triglyceride content and myocardial function.

Methods
Ten lean healthy men were included in this prospective intervention study. Three-day partial (471 kcal/day) and complete starvation was performed. Plasma levels of NEFA, myocardial and hepatic triglyceride content, and myocardial function were calculated.

Results
Plasma NEFA increased from 0.6 ± 0.4 to 1.2 ± 0.4 and to 1.9 ± 0.7 mmol/l, after partial and complete starvation, respectively (P < 0.001). Myocardial triglyceride content increased from 0.35 ± 0.14 to 0.59 ± 0.27, and 1.26 ± 0.49%, respectively (P < 0.01). The ratio between the early diastole and atrial contraction decreased from 2.2 ± 0.4 to 2.1 ± 0.4 (P = 0.7) and 1.8 ± 0.4, respectively (P < 0.01), and diastolic early deceleration decreased from 3.4 ± 0.7 to 2.9 ± 0.5 and 2.8 ± 0.9 ml/s² × 10⁻³, respectively (P < 0.05). Hepatic triglyceride content decreased after partial starvation (from 2.23 ± 2.24 to 1.43 ± 1.33%; P < 0.05) but did not change upon complete starvation.

Conclusion
Progressive caloric restriction induces a dose-dependent increase in myocardial triglyceride content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic triglyceride content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous triglyceride stores is tissue-specific.
Almost all endogenous triglycerides are stored in adipose tissue to accommodate discrepancies between whole body fat uptake and fat oxidation. However, a very small proportion is stored in non-adipose tissues like the heart, the liver, and skeletal muscle, especially in obesity and type 2 diabetes mellitus. There are indications that this storage of triglycerides in non-adipose tissues is not merely an inert phenomenon but is associated with more or less subtle physiological changes in organ-specific functioning.

In animal models there is an inverse relation between myocardial triglyceride content and myocardial function. For example, myocardial lipid accumulation is associated with a decrease in left ventricular systolic function in obese Zucker rats, and treatment with thiazolidinediones reduces myocardial triglyceride content and improves left ventricular function. The underlying mechanisms of the decrease in left ventricular function are complex and are related to effects of fatty acid (FA) derivatives, like fatty acyl-coenzyme A, ceramides, and diacylglycerol.

High plasma concentrations of non-esterified fatty acids (NEFA) may result in excessive FA uptake in non-adipose tissues, such as the liver and heart, which may affect normal organ function. However, in humans the relation between myocardial triglyceride accumulation and myocardial function was difficult to study by non-invasive methods because measurement of myocardial triglyceride content is challenging due to artifacts induced by cardiac and respiratory motion. Recently, proton magnetic resonance spectroscopy (1H-MRS) of the heart was developed that enables the measurement of myocardial triglyceride content in humans in vivo. Using this method, Reingold et al. documented that fasting for 48 hours increases plasma NEFA levels and myocardial triglyceride content in healthy subjects, whereas myocardial triglyceride content did not change after a single high-fat meal.

In another, cross-sectional study, Kankaanpää et al. showed that increased levels of plasma NEFA in obese subjects correlate positively with myocardial triglyceride content and inversely with myocardial function. However, both studies did not address the relation between myocardial function in relation to myocardial triglyceride content within the same subjects. In a recent study, we documented that the use of a very low calorie diet increases plasma NEFA and myocardial triglyceride content, and was associated with a decrease in myocardial diastolic function. Therefore, it appears that myocardial triglyceride content is not fixed but varies within the same subject according to physiological conditions. It is yet unknown whether our recent findings of myocardial flexibility can be extrapolated when caloric restriction is progressively increased.

Therefore, the aim of the present study was to extend the conditions of partial caloric restriction to complete caloric restriction, i.e. complete starvation. For this purpose we compared baseline observations with those obtained after 3-day partial starvation (471 kcal/day) and after 3-day complete starvation with respect to plasma levels of NEFA, myocardial triglyceride content, myocardial function, and hepatic triglyceride content.
METHODS

Subjects
There were 10 non smoking, healthy men included in this study (age; mean ± SD: 23.7 ± 4.7 years, range 20.8 – 36.0 years; body mass index [BMI]: 23.6 ± 0.9 kg/m²). Women were excluded because the hormonal status or contraceptive use may affect lipid metabolism.16 The study population was partly based on a previous cohort.15 In each subject, medical history was obtained, and physical examination was performed. An electrocardiogram (ECG) was made during the first visit. Subjects with any aberrations on the ECG were excluded. In addition, a 75-g oral glucose tolerance test to exclude subjects suffering from diabetes mellitus was performed.17 Other exclusion criteria were: obesity (BMI > 30 kg/m²); liver disease (increased plasma levels of alanine aminotransferase, aspartate aminotransferase, and/or γ-glutamyl transferase ± 2 SD above the reference value of our institution); renal disease (defined by plasma creatinine levels ± 2 SD above the reference value of our institution); use of any medication; and a history of (congenital) heart disease. Specifically, subjects with prior or present coronary artery disease (based on medical history) or hypertension (defined as sitting systolic blood pressure > 130 mmHg and/or diastolic blood pressure > 85 mmHg) were excluded. Written informed consent was obtained from all participants before the study. The local ethics committee approved the study.

Study design
Baseline measurements were made, while subjects followed a normal diet but abstained from alcohol for 3 days (mean intake 2065 kcal/day). Subjects were admitted 4 hours after the last meal for measurement of plasma concentrations of glucose, insulin, and lipids, and for evaluation by magnetic resonance imaging (MRI) and MRS. The second measurement was performed after a 3-day period of partial caloric restriction (471 kcal/day; Modifast Intensive, Nutrition & Sante Benelux, Breda, the Netherlands). The third measurement was performed after a 3-day period of complete starvation (0 kcal/day, only water was allowed), after which subjects were again admitted for blood sampling and MRI/ MRS evaluation. Plasma concentrations of NEFA and insulin were used to assess study compliance.18 Between all study occasions, a washout period with a minimum of 14 days was acquired,19 and the sequence of the second and third measurement was determined by balanced assignment.

1H-magnetic resonance spectroscopy of liver and heart
All MRI/ MRS studies were performed with the use of a 1.5T whole-body MR scanner (Gyroscan ACS/ NT15; Philips, Best, Netherlands) with subjects in supine position at rest. Localized single-voxel (2 × 2 × 2 cm for the liver and 2 × 4 × 1 cm for the heart)
spectra were recorded using a body coil for radiofrequency transmission and a surface coil (17 cm diameter) for signal receiving. For the heart, the spectral volume was placed in the interventricular septum on four-chamber and short-axis images at end-systole, avoiding contamination with epicardial fat (Figure 5.1). Data collection was double triggered using ECG-triggering and navigator echoes for compensation of respiratory motion as described earlier. For the liver, voxel sites were matched at both study occasions, carefully avoiding gross vascular structures. To detect the weak lipid signals, water-suppressed spectra with 128 averages for the heart and 64 for the liver were collected. Spectral parameters were: a repetition time (TR) of at least 3000 ms, echo time (TE) = 26 ms, and a total of 1024 data points were collected using a 1000-Hz spectral width. In the same voxel, using the same parameters except for a TR of 10 seconds, unsuppressed spectra with 4 averages were collected. Spectra were analyzed in the time domain, using Java-based MR user interface software (version 2.2) and prior knowledge files, as described earlier. Peak estimates of lipid resonances of myocardial triglycerides at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (triglyceride content, triglyceride/water ×100).

Figure 5.1
Myocardial spectroscopic volume. Localization of the myocardial spectral voxel in the four-chamber (A) and short-axis views (B).

Magnetic resonance imaging of the heart
Imaging of the heart was performed using a body coil for radiofrequency transmission and a five-element synergy coil for signal receiving. To assess systolic function, the heart was imaged from apex to base with 12–14 imaging levels (dependent on the heart size) in short-axis view using an ECG-triggered, sensitivity-encoding balanced steady-state free procession sequence. Imaging parameters were: field of view = 400 × 400 mm, matrix size = 256 × 256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°,
TE = 1.7 ms, and TR = 3.4 ms. Temporal resolution was 25–39 ms. End-diastolic and end-systolic images were identified on all slices, and endocardial contours were drawn using MASS post-processing software (Medis, Leiden, the Netherlands) as described previously.\textsuperscript{21} Left ventricular ejection fraction (LVEF) was calculated for the assessment of systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding (Venc) was performed to measure blood flow across the mitral valve for the determination of left ventricular diastolic function.\textsuperscript{22,23} Imaging parameters included the following: TE = 5 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, Venc = 100 cm/s, and scan percentage = 80%. Flow velocities in early diastole (E) and at atrial contraction (A) were measured, and their peak flow ratio was calculated (E/A ratio) using the FLOW analytical software package (Medis, Leiden, the Netherlands) by defining a region of interest on the modulus images in all cardiac phases. Furthermore, the mean deceleration of the E wave and an estimation of left ventricular filling pressures (E/Ea)\textsuperscript{24} were measured. All spectroscopic and functional analyses were performed by an experienced observer, blinded to the interventions. During MRI, blood pressure and heart rate were measured twice with an automatic device (Dinamap DPC100X; Freiburg, Germany) and averaged for analysis.

Assays
Plasma glucose, total cholesterol, and triglycerides were measured on a fully automated P800 analyzer (Roche, Almere, the Netherlands) and plasma insulin on an Immulite 2500 random access analyzer with a chemoluminescence immunoassay (Diagnostic Products Corp., Los Angeles, CA). Coefficients of variation were less than 2% for plasma glucose, total cholesterol, and triglycerides, and less than 5% for insulin. Plasma NEFA were measured using a commercial kit (FFA-C; Wako Chemicals, Neuss, Germany).

Statistical analysis
All statistical analyses were performed using SPSS (version 12.0; SPSS, Chicago, IL). Statistical comparisons among the three physiological conditions were made by repeated measures ANOVA. Pearson r values were used for correlation analysis. Data are shown as mean ± standard deviation (SD). \( P < 0.05 \) (two-tailed) was considered significant. Based on a previous report, we expected a decrease in diastolic early deceleration. Therefore, \( P < 0.05 \) (one-tailed) was considered significant for this parameter.\textsuperscript{15}
RESULTS

Metabolic effects of progressive caloric restriction

Subject characteristics at baseline, after partial starvation, and after complete starvation are shown in Table 5.1. Postabsorptive plasma glucose levels decreased from 5.0 ± 0.3 mmol/l at baseline to 4.3 ± 0.4 mmol/l after partial (P = 0.001) and to 3.9 ± 0.5 mmol/l after complete starvation (P < 0.001). This was associated with a dose-dependent decrease in plasma insulin levels. Simultaneously, plasma concentrations of NEFA increased dose-dependently from 0.6 ± 0.4 to 1.2 ± 0.4 mmol/l after partial (P < 0.001) and to 1.9 ± 0.7 mmol/l after complete starvation (P < 0.001). Plasma triglyceride levels decreased after partial starvation (from 1.3 ± 0.4 to 0.9 ± 0.3 mmol/l (P = 0.009) but did not change upon complete starvation (P = 0.677). Total cholesterol increased from 5.0 ± 1.3 mmol/l at baseline to 5.1 ± 1.4 mmol/l after partial (P = 0.810) and to 5.9 ± 1.8 mmol/l after complete starvation (P = 0.005).

<table>
<thead>
<tr>
<th>Plasma concentrations</th>
<th>Baseline</th>
<th>Partial starvation</th>
<th>Complete starvation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.0 ± 0.3</td>
<td>4.3 ± 0.4*</td>
<td>3.9 ± 0.5*</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>10.1 ± 5.3</td>
<td>8.0 ± 3.7</td>
<td>3.0 ± 1.8*</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>0.6 ± 0.4</td>
<td>1.2 ± 0.4†</td>
<td>1.9 ± 0.7†</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.3 ± 0.4</td>
<td>0.9 ± 0.3*</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.0 ± 1.3</td>
<td>5.1 ± 1.4</td>
<td>5.9 ± 1.8*</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Blood samples were collected 4 hours after the last meal.

* P < 0.01 and † P < 0.001 vs. baseline.

Effects of progressive caloric restriction on myocardial and hepatic triglyceride content

Myocardial triglyceride content increased dose dependently from 0.35 ± 0.14% at baseline to 0.59 ± 0.27% after partial (P = 0.006) and to 1.26 ± 0.49% after complete starvation (P < 0.001; Figure 5.2). Hepatic triglyceride content correlated with BMI at baseline (r = 0.67; P = 0.033). Hepatic triglyceride content significantly decreased after partial starvation (from 2.24 ± 2.24 to 1.43 ± 1.33%; P = 0.031), whereas it did not change after complete starvation (2.54 ± 2.53%; P = 0.378; Figure 5.3).

Effects of progressive caloric restriction on myocardial function

Systolic and diastolic blood pressure, heart rate, and myocardial LVEF did not change significantly during/after partial and complete starvation, compared with baseline (Table 5.2). Furthermore, estimated left ventricular filling pressures were unchanged after partial (8.8 ± 3.8; P = 0.742) and complete starvation (8.2 ± 2.5; P = 0.299) compared with baseline (9.3 ± 2.6). Diastolic E/A ratio decreased dose dependently from 2.2 ±
Figure 5.2
Myocardial triglyceride content at baseline and after partial and complete starvation. Typical proton spectra of myocardial triglyceride content of one subject at baseline and after partial and complete starvation scaled relative to baseline (A) and individual changes in myocardial triglyceride content upon complete starvation (n = 10 [B]). Vertical lines represent mean ± standard deviation.

TG = triglyceride.

* P < 0.01 vs. baseline.
0.4 at baseline to 2.1 ± 0.4 after partial starvation (P = 0.687) and to 1.8 ± 0.4 after complete starvation (P = 0.005). E deceleration decreased dose dependently from 3.4 ± 0.7 ml/s² × 10⁻³ at baseline to 2.9 ± 0.5 ml/s² × 10⁻³ after partial (P = 0.036) and to 2.8 ± 0.9 ml/s² × 10⁻³ after complete starvation (P = 0.032).

Figure 5.3
Hepatic triglyceride content at baseline, and after partial and complete starvation. Individual changes in hepatic triglyceride content upon complete starvation (n = 10). Vertical lines represent mean ± standard deviation.
* P < 0.05 vs. baseline.

| Table 5.2 Effects of progressive caloric restriction on myocardial function |
|-------------------------------------------------|--------------------|--------------|
| Systolic blood pressure (mmHg)                  | Baseline           | Partial starvation | Complete starvation |
| 120 ± 10                                        | 118 ± 9            | 122 ± 12       |
| Diastolic blood pressure (mmHg)                 | 64 ± 7             | 62 ± 7         | 61 ± 5           |
| Heart rate (bpm)                                | 62 ± 13            | 59 ± 10        | 65 ± 10          |
| Left ventricular ejection fraction (%)           | 60 ± 4             | 59 ± 4         | 60 ± 6           |
| E/A                                            | 2.2 ± 0.4          | 2.1 ± 0.4      | 1.8 ± 0.4*       |
| E deceleration (ml/s² × 10⁻³)                   | 3.4 ± 0.7          | 2.9 ± 0.5†     | 2.8 ± 0.9†       |

Values are mean ± standard deviation.
* P < 0.01, † P < 0.05 vs. baseline.
DISCUSSION

This study demonstrates that progressive caloric restriction increases myocardial triglyceride content in lean healthy men. This increase is paralleled by decreased diastolic myocardial function. In addition, the results document a dose-dependent effect between the degree of caloric restriction and the myocardial effects. These observations point to physiological variations in myocardial triglyceride content and diastolic function. The effect of caloric restriction on redistribution of endogenous triglyceride stores is tissue-specific because we demonstrated differential effects of partial and complete starvation on liver triglyceride content.

Different degrees of starvation were associated with a considerable increase in plasma NEFA levels, in accordance with previous observations. These increased NEFA levels reflect increased lipolysis of triglyceride content in adipose tissue. Apparently, during starvation myocardial FA uptake exceeds the requirements of myocardial FA oxidation, resulting in increased triglyceride stores. Moreover, progressive caloric restriction has dose-dependent effects on myocardial triglyceride accumulation and myocardial function. However, a causal relationship between myocardial triglyceride content and myocardial function cannot be derived from the present data.

Our data are supported by animal experiments. In those studies excessive exposure of the myocardium to plasma NEFA is accompanied by increased storage of myocardial triglycerides, resulting in the production of fatty acid intermediates, and ultimately in deteriorations in myocardial function. Accordingly, it has been suggested that in obese subjects, subclinical diastolic dysfunction is due to changes in myocardial metabolism. Kankaanpää et al. reported that alterations in left ventricular function in moderate obese subjects are associated with increased myocardial triglyceride content, compared with lean subjects. Moreover, Szczepaniak et al. showed increased myocardial triglyceride content in overweight and obese subjects, which was accompanied by increased left ventricular mass. In accordance with our study, Reingold et al. documented that short-term fasting leads to myocardial triglyceride accumulation, although they did not document effects on myocardial function. The current results, documenting dose-dependent effects of caloric restriction on levels of plasma NEFA, myocardial triglyceride content, and diastolic function, extend these findings and support the general concept that increased myocardial triglyceride content is associated with decreased myocardial function. Alternatively, starvation profoundly alters endogenous metabolic regulation and other, yet undefined, metabolic effects than merely increased levels of plasma NEFA and myocardial triglyceride content, which may be involved in explaining the reduction in myocardial diastolic function. For example, caloric restriction might change calcium homeostasis in the myocardium, which affects myocardial diastolic function.
Transmitral flow velocities are load dependent and can be affected by changes in intravascular volume. However, estimated left ventricular filling pressures were unchanged upon progressive caloric restriction. Therefore, we believe that the observed change in transmitral flow patterns results from a change in the relaxation of the left ventricle. Caloric restriction enhances adipose tissue lipolysis, reflected in increased levels of plasma NEFA, due to reduced insulin levels. Similar to our results in the heart, others found corresponding results of increased triglyceride content of skeletal muscle after fasting. Starvation affects more parameters of lipid metabolism because plasma NEFA stimulate the hepatic production of very low density lipoprotein (VLDL), which is an important supplier for triglycerides to the heart. Plasma NEFA levels also increase during starvation and most likely will contribute to increased myocardial triglyceride levels. However, the relative contribution of albumin-bound fatty acids vs. fatty acids derived from VLDL-triglyceride to myocardial triglyceride stores during caloric restriction cannot be derived from the present data. We found a correlation between hepatic fat content and BMI, in accordance with previous observations. However, despite the increase in the flux of plasma NEFA to the liver, considering the increased plasma NEFA levels, hepatic triglyceride content was decreased after partial starvation but was unchanged after complete starvation. In line with our results, Westerbacka et al. previously documented that a low fat diet in moderately obese women decreases hepatic triglyceride content. Because hepatic triglyceride content is tightly regulated by the balance of hepatic fatty acid uptake, hepatic fatty acid oxidation, and output of VLDL-triglyceride particles, it is possible that this hepatic balance between fatty acid uptake and triglyceride output is differentially affected by partial and complete starvation. Nonetheless, our data indicate that progressive caloric restriction differentially affects tissue-specific stores of triglycerides in heart and liver, and show that myocardial triglyceride content and myocardial function vary depending on nutritional conditions, at least with respect to progressive degrees of starvation. Additional studies are required to elucidate to which extent these results can be extrapolated to clinically relevant conditions like type 2 diabetes mellitus and obesity. In conclusion, progressive caloric restriction induces a dose-dependent increase in myocardial triglyceride content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic triglyceride content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous triglyceride stores is tissuespecific, at least in lean healthy men.
REFERENCES


