Chapter 4

Progressive Caloric Restriction Induces Dose-dependent Changes in Myocardial Triglyceride Content and Diastolic Function in Healthy Men


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

Materials and methods: This study included 10 lean healthy men. Three-day partial (471 kcal/d) and complete starvation was performed. Plasma levels of NEFAs, myocardial and hepatic TG content (\(^1\)H magnetic resonance (MR) spectroscopy, and myocardial function (MR imaging) were calculated.

Results: Plasma NEFAs increased from mean ± standard deviation 0.6 ± 0.4 mmol/l to 1.2 ± 0.4 and to 1.9 ± 0.7 mmol/l, after partial and complete starvation, respectively (\(P < 0.001\)). Myocardial TG 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 from 3.4 ± 0.7 ml/s\(^2\) \times 10\(^{-3}\) to 2.9 ± 0.5 and 2.8 ± 0.9 ml/s\(^2\) \times 10\(^{-3}\), respectively (\(P < 0.05\)). Hepatic TG 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.

Conclusions: Progressive caloric restriction induces a dose-dependent increase in myocardial TG content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic TG content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous TG stores is tissue-specific.
INTRODUCTION

Almost all endogenous triglycerides (TGs) 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 (1), the liver (2), and skeletal muscle (3), especially in obesity and type 2 diabetes mellitus. There are indications that this storage of TG in non-adipose tissues is not merely an inert phenomenon but is associated with more or less subtle physiological changes in organ-specific functioning (4-8). In animal models there is an inverse relation between myocardial TG 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 TG content and improves left ventricular function (7). 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 (4;6;8).

High plasma concentrations of non-esterified fatty acids (NEFAs) may result in excessive FA uptake in non-adipose tissues, such as the liver and the heart, which may affect normal organ function (6;7). However, in humans the relation between myocardial TG accumulation and myocardial function was difficult to study by non-invasive methods, as measurement of myocardial TG content is challenging due to artifacts induced by cardiac and respiratory motion. Recently, hydrogen 1 magnetic resonance spectroscopy ($^1$HMRS) of the heart was developed which enables to measure myocardial TG content in humans in vivo (1;9-12). Using this method, Reingold et al. documented that fasting for 48 hours increases plasma NEFA levels and myocardial TG content in healthy subjects, whereas myocardial TG content did not change after a single high-fat meal (13). In another, cross-sectional study, Kankaanpää et al. showed that increased levels of plasma NEFAs in obese subjects correlate positively with myocardial TG content and inversely with cardiac function (11). However, both studies did not address the relation between myocardial function in relation to myocardial TG content within the same subjects. In a recent study we documented that the use of a very low-calorie diet increases plasma NEFAs and myocardial TG content, associated with a decrease in myocardial diastolic function (14). Therefore, it appears that myocardial TG 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 days of a partial starvation (471 kcal/day) and after 3 days of complete starvation with respect to plasma levels of NEFAs, myocardial TG content, myocardial function and hepatic TG content.
MATERIALS AND METHODS

Subjects
There were ten non-smoking, healthy men included in this study (age; mean ± standard deviation: 23.7 ± 4.7 years, range 20.8-36.0 years, body mass index (BMI): 23.6 ± 0.9 kg/m²). Women were excluded, as the hormonal status or contraceptive use may affect lipid metabolism (15). The study population was partly based on a previous cohort (14). 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 two-hour 75 g oral glucose tolerance test was performed in the fasted state, to exclude subjects suffering from diabetes mellitus (16). Other exclusion criteria were: obesity (BMI > 30 kg/m²), liver disease (increased plasma levels of alanine aminotransferase, aspartate aminotransferase and/or gamma-glutamyl transferase > 2 standard deviations above the reference value of our institution), renal disease (defined by plasma creatinine levels > 2 standard deviations 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. From all participants written informed consent was obtained prior to the study. The local ethics committee approved the study.

Study design
The study consisted of 3 conditions. 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 (MR) imaging and 1HMRS. The second measurement was performed after a 3-day period of partial caloric restriction (471 kcal/day, Modifast Intensive, Nutrition & Santé 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 MR imaging and 1HMRS evaluation. Plasma concentrations of NEFAs and insulin were used to assess study compliance (17). Between all study occasions a washout period with a minimum of 14 days was acquired (18), and the sequence of the second and third occasions was determined by balanced assignment.

1H magnetic resonance spectroscopy of the liver and the heart
All MR imaging and 1HMRS measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) in the supine position. 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 (Ø 17 cm) 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 4.1). Data collection was double-triggered by using ECG triggering and navigator echoes for compensation of respiratory motion as described earlier (12). For the liver, voxel sites were matched at both study occasions, carefully avoiding blood vessels and bile ducts. To detect 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 3000 ms, echo time (TE) of 26 ms and 1024 data points over 1000-Hz spectral width. In the same voxel, using the same parameters except for a repetition time of 10000 ms, unsuppressed spectra with 4 averages were collected. Spectra were analyzed in the time domain, using Java-based MR user interface software and prior knowledge files (jMRUI version 2.2 (19)), as described earlier (12). Peak estimates of lipid resonances of myocardial TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TG content, TGs/water ×100).

**Figure 4.1. Myocardial spectroscopic volume.**
Localization of the myocardial voxel in the four-chamber (A) and short-axis (B) views.

**Magnetic resonance imaging of the heart**
Imaging of the heart was performed using a body coil for radiofrequency transmission and a 5 elements synergy coil for signal receiving. In order to assess systolic function, the heart was imaged from apex to base with 12 to 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 a field of view of $400 \times 320$ mm, a matrix size of $256 \times 256$, a slice thickness of 10 mm, a slice gap of 0 mm, a flip angle of 35°, a TE of 1.7 ms and a TR of 3.4 ms. Temporal resolution was 25 to 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 (20). Left ventricular ejection fraction
(LVEF) was calculated for assessment of systolic function. Furthermore, an ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of left ventricular diastolic function (21;22). Imaging parameters included the following: a TE of 4.8 ms, a TR of 14 ms, a flip angle of 20°, a slice thickness of 8 mm, a field of view of 350 mm², a matrix size of 256 × 256, a velocity encoding of 100 cm/s and a scan percentage of 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) (23) were measured. All spectroscopic and functional analyses were performed by an experienced observer, blinded to the interventions. During MR imaging, blood pressure and heart rate were measured twice with an automatic device (Dinamap DPC100X, Freiburg, Germany) and averaged for analysis.

**Assays**

Glucose, total cholesterol (TC) and TGs were measured on a fully automated P800 analyzer (Roche, Almere, The Netherlands) and insulin on a Immulite 2500 random access analyzer with a chemoluminescence immunoassay (DPC, Los Angeles, CA, USA). Coefficients of variation were < 2% for glucose, TC and TG, and < 5% for insulin. Plasma NEFAs were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany).

**Statistical analysis**

All statistical analyses were performed using SPSS, version 12.01 (SPSS Inc., Chicago, Ill, USA). Statistical comparisons between 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. P < 0.05 (two-tailed) was considered significant. Based on previous report we expected a decrease in diastolic early deceleration. Therefore P < 0.05 (one-tailed) was considered to be significant for this parameter (14).

**RESULTS**

**Metabolic effects of progressive caloric restriction**

Subject characteristics at baseline, after partial starvation, and after complete starvation are shown in Table 4.1. 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 NEFAs increased dose-dependently from 0.6 ± 0.4 mmol/l 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 TG levels decreased after partial starvation (from 1.3 ± 0.4 mmol/l to 0.9 ± 0.3 mmol/l ($P = 0.009$), but did not change upon complete starvation ($P = 0.677$). TC 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 ($P = 0.005$).

### Effects of progressive caloric restriction on myocardial and hepatic triglyceride content

Myocardial TG 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 4.2). Hepatic TG content correlated with BMI at baseline ($r = 0.67$, $P = 0.033$). Hepatic TG 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 4.3).

### 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 to baseline (Table 4.2). 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 to baseline (9.3 ± 2.6). Diastolic E/A ratio decreased dose-dependently from 2.2 ± 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/s$^2$ $×$ 10$^{-3}$ at baseline to 2.9 ± 0.5 $×$ 10$^{-3}$ ml/s$^2$ after partial ($P = 0.036$) and to 2.8 ± 0.9 after complete starvation ($P = 0.032$).

### Table 4.2. Effects of progressive caloric restriction on myocardial function.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Partial starvation</th>
<th>Complete starvation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>120 ± 10</td>
<td>118 ± 9</td>
<td>122 ± 12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>64 ± 7</td>
<td>62 ± 7</td>
<td>61 ± 5</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>62 ± 13</td>
<td>59 ± 10</td>
<td>65 ± 10</td>
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<tr>
<td>LVEF (%)</td>
<td>60 ± 4</td>
<td>59 ± 4</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>2.2 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>1.8 ± 0.4$^*$</td>
</tr>
<tr>
<td>E deceleration (ml/s$^2$ $×$ 10$^{-3}$)</td>
<td>3.4 ± 0.7</td>
<td>2.9 ± 0.5$^{*}$</td>
<td>2.8 ± 0.9$^{*}$</td>
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* $P < 0.01$, † $P < 0.05$ vs baseline. Data are mean ± standard deviation.

LVEF = left ventricular ejection fraction, E = early diastolic wave, A = atrial diastolic wave.
Figure 4.2. Myocardial triglyceride content at baseline and after partial and complete starvation. Typical $^1$H spectra of myocardial triglyceride (TG) content of one subject at baseline and after partial and complete starvation scaled relative to baseline (A) and individual changes in myocardial TG content upon complete starvation ($n = 10$) (B). Vertical lines represent mean ± standard deviation, * $P < 0.01$ vs baseline. ppm = parts per million.
DISCUSSION

This study demonstrates that progressive caloric restriction increases myocardial TG 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 TG content and diastolic function. The effect of caloric restriction on redistribution of endogenous TG stores is tissue-specific, since we demonstrated differential effects of partial and complete starvation on liver TG content. Different degrees of starvation were associated with a considerable increase in plasma NEFA levels, in accordance with previous observations (24;25). These increased NEFA levels reflect increased lipolysis of TG content in adipose tissue. Apparently, during starvation myocardial FA uptake exceeds the requirements of myocardial FA oxidation, resulting in increased TG stores. Moreover, progressive caloric restriction has dose-dependent effects on myocardial TG accumulation and myocardial function. However, a causal relationship between myocardial TG content and myocardial function can not be derived from the present data.

Our data are supported by animal experiments. In those studies excessive exposure of the myocardium to plasma FA is accompanied by increased storage of myocardial TGs, resulting
in the production of FA intermediates, and ultimately deteriorations in myocardial function (7;26;27). Accordingly, it has been suggested that in obese subjects, subclinical diastolic dysfunction is due to changes in myocardial metabolism (28-31). Kankaanpää et al. reported that alterations in left ventricular function in moderate obese subjects are associated with increased myocardial TG content, compared to lean subjects (11). Moreover, Szczepaniak et al. showed increased myocardial TG content in overweight and obese subjects, which was accompanied by increased left ventricular mass (1). In accordance with our study, Reingold et al. documented that short-term fasting leads to myocardial TG accumulation, although they did not document effects on myocardial function (13). The current results, documenting dose-dependent effects of caloric restriction on levels of plasma NEFAs, myocardial TG content and diastolic function, extent these findings and support the general concept that increased myocardial TG content is associated with decreased myocardial function (32). Alternatively, starvation profoundly alters endogenous metabolic regulation and other, yet undefined, metabolic effects than merely increased levels of plasma NEFAs and myocardial TG content, which may be involved to explain the reduction in myocardial diastolic function. For example, caloric restriction might change calcium homeostasis in the myocardium (33), which affects myocardial diastolic function (34).

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 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 NEFAs, due to reduced insulin levels. Similar to our results in the heart, others found corresponding results of increased TG content of skeletal muscle after fasting (18;24;25). Starvation affects more parameters of lipid metabolism, because plasma NEFAs stimulate the hepatic production of very low-density lipoprotein (VLDL), which is an important supplier for TG to the heart (35;36). Plasma NEFA levels also increase during starvation and most likely will contribute to increased myocardial TG levels. However, the relative contribution of albumin-bound fatty acids vs fatty acids derived from VLDL-TGs to myocardial TG stores during caloric restriction can not be derived from the present data.

We found a correlation between hepatic fat content and BMI, in accordance with previous observations (2;37). However, despite the increase in the flux of plasma NEFAs to the liver, considering the increased plasma NEFA levels, hepatic TG 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 TG content (38). Because hepatic TG content is tightly regulated by the balance of hepatic FA uptake, hepatic FA oxidation and output of VLDL-TG particles, it is possible that this hepatic balance between FA uptake and TG output is differentially affected by partial and complete starvation. Nonetheless, our data indicate that progressive caloric restriction differentially affects tissue-specific stores of TGs in heart and liver, and prove that myocardial TG 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.

**CONCLUSIONS**

In conclusion, progressive caloric restriction induces a dose-dependent increase in myocardial TG content and a dose-dependent decrease in diastolic function in lean healthy men. Hepatic TG content showed a differential response to progressive caloric restriction, indicating that redistribution of endogenous TG stores is tissue-specific, at least in lean healthy men.
REFERENCES


