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Brief Report

Skeletal muscle extracellular matrix remodeling after short-term overfeeding in healthy humans

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ABSTRACT

Background. Skeletal muscle extracellular matrix (ECM) remodeling has been proposed as a feature of the pathogenic milieu associated with obesity and metabolic dysfunction. The aim of the current study was to examine the timeline of this response and determine whether 3 and 28 days of overfeeding alters markers of ECM turnover.

Methods. Forty healthy individuals were overfed by 1250 kcal/day for 28 days. Hyperinsulinemic–euglycemic clamps and abdominal fat distribution were performed at baseline and day 28 of overfeeding and skeletal muscle biopsies taken at baseline, day 3 and day 28. mRNA expression (COL1a1, COL3a1, MMP2, MMP9, TIMP1, CD68, Integrin) was performed in 19 subjects that consented to having all biopsies performed and microarray analysis was performed in 8 participants at baseline and day 28.

Results. In the whole cohort, body weight increased by 0.6 ± 0.1 and 2.7 ± 0.3 kg at days 3 and 28 (both $P < 0.001$), respectively. Glucose infusion rate during the hyperinsulinemic–euglycemic clamp decreased from 54.8 ± 2.8 at baseline to 50.3 ± 2.5 μmol/min/kg FFM at day 28 of overfeeding ($P = 0.03$). Muscle COL1 and COL3 and MMP2 mRNA levels were significantly higher 28 days after overfeeding (all $P < 0.05$), with no significant changes in MMP9, TIMP1, CD68 and integrin expression. Microarray based gene set tests revealed that pathways related to ECM receptor interaction, focal adhesion and adherens junction were differentially altered.

Conclusions. Skeletal muscle ECM remodeling occurs early in response to over-nutrition with as little as 3% body weight gain. Our findings contribute to the growing evidence linking muscle ECM remodeling and accumulation as another sequela of obesity-related insulin resistance.

Abbreviations: COL, collagen; ECM, extracellular matrix; GIR, glucose infusion rate; GST, gene set test; MMP, matrix metalloproteinase; SPARC, secreted protein acidic and rich in cysteine; TIMP, tissue inhibitor of metalloproteinase.

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1. Introduction

The extracellular matrix (ECM) is essential for tissue architecture and regulating intercellular communication and undergoes substantial remodeling as a result of injury and repair. Significant changes in the ECM are also observed as a result of obesity. In subcutaneous adipose tissue, obesity is associated with excess collagen deposition and impaired degradation, reduced tissue plasticity and adipocyte dysfunction [1,2]. Mounting evidence suggests that the skeletal muscle ECM is also dysregulated during energy excess and is associated with diet-induced insulin resistance [3–6]. Collagen abundance is higher in *vastus lateralis* biopsies from obese insulin-resistant humans and in diet-induced obese mice and insulin resistance and muscle collagen accumulation is reversed after pharmacological or genetic manipulation targeting the muscle ECM [3–5]. Furthermore, an earlier overfeeding study performed in lean, healthy men found dramatic upregulation of mRNA levels of genes related to ECM accumulation (collagens I, III, IV, V, VI and SPARC) in muscle after 10% weight gain [7]. The increases in muscle ECM gene expression paralleled increases in lean tissue mass, suggesting that these changes may be indicative of a physiological response to nutrient excess, rather than a pathological fibrotic tissue response [7]. The aim of this study was to examine the timeline of this response and determine whether 3 and 28 days of overfeeding alters markers of ECM turnover.

2. Methods

2.1. Participants and Overfeeding Intervention

The study cohort, intervention and metabolic testing have been described previously [8]. Briefly, 40 healthy individuals [20 women (5 post-menopausal) and 20 men, mean age 37 years (range = 21–59 years)] completed 28 days of an overfeeding protocol outlined below. Diet at baseline was provided from day −3 to day −1 at calculated energy requirements with a nutrient composition of 30% fat, 15% protein, 55% carbohydrate and days 0–3 and 25–28 at 1250 kcal/day above baseline energy requirements with a nutrient composition of 45% fat, 15% protein and 40% carbohydrate. Overfeeding between days 3 and 25 was achieved by supplementing the baseline diet with energy-dense snacks and a liquid oil-based supplement to increase energy by +1250 kcal/d that were provided to the participants. Weight gain and study compliance were monitored weekly by the research nurse and dietician. The study protocol was approved by the Human Research and Ethics Committee at St Vincent's Hospital, Sydney and subjects provided informed written consent before commencing the study.

2.2. Metabolic Testing and Body Composition

Participants attended the Clinical Research Facility at 8am after a 12-h fast at baseline and at days +3 and +28 of overfeeding. Baseline and +28 day visits were identical and included a 2-h hyperinsulinemic–euglycemic clamp (60 mU/m^2/min) and fat-mass and fat-free mass measurement by DXA. Blood samples were taken at baseline and at days +3 and +28 of overfeeding, with glucose measured by YSI Analyzer (YSI Life Sciences) and serum insulin by RIA (Linco Research, St Charles).

2.3. Muscle Gene Expression and Microarray Pathway Analyses

Vastus lateralis biopsies were performed as previously described [9]. Total RNA was extracted from 25mg of muscle tissue using TRIzol reagent (Invitrogen, Carlsbad, CA) and RNA integrity and concentration were assessed by spectrophotometry (Nanodrop Technologies, USA). cDNA was synthesized using Qiagen (Germany) and Recombinant RNAsin RNase inhibitor (Promega, Madison, WI) according to kit instructions. RT-PCR gene expression was performed in 19 participants who had all 3 biopsies performed (baseline, days +3 and +28) and that had sufficient biopsy material for analyses. Seven genes (COL1a1, COL3a1, MMP2, MMP9, TIMP1, CD68, Integrin$\alpha 2\beta 1$) were selected from previously published literature on extracellular matrix remodeling [6,7] and measured using gene specific primer-probes from Taqman. Samples were run in duplicate on an ABI Prism 7500 system (Applied Biosystems, Darmstadt, Germany) and normalized to 18S, which was not different before and after overfeeding ($P = 0.11$).

Affymetrix microarrays were performed at the Ramaciotti Centre for Gene Function Analysis, University of New South Wales, Sydney in a subset of muscle samples from 8 individuals before and 28 days after overfeeding. Selection was based on individuals who had the largest (n = 4) *vs.* smallest change in glucose infusion rate (GIR) during the hyperinsulinemic–euglycemic clamp (n = 4), had gained at least 2 kg after overfeeding and had sufficient muscle biopsy available. Microarray analyses are described in Supplementary Data.

2.4. Statistical Analysis

Data are presented as mean ± SEM. Statistics were performed using R Version 3.1.2. Mixed models were performed to examine the effects of time (baseline, day 3, day 28) on overfeeding, anthropometry and metabolism and muscle gene expression with post-hoc tests performed using Tukey contrasts.

3. Results

3.1. Anthropometric and Metabolic Effects of Overfeeding

As previously reported [9], overfeeding led to an average weight gain of 0.6 ± 0.1 at day 3 and 2.7 ± 0.3 kg at day 28 in the whole cohort (Table 1). Percent body fat and circulating glucose and insulin levels were significantly increased after 28 days of overfeeding. Likewise, insulin resistance measured by homeostasis model of assessment of insulin resistance (HOMA-IR) increased significantly and GIR during the hyperinsulinemic–euglycemic clamp decreased significantly with overfeeding; all these responses were not different between men and women. Anthropometric and metabolic effects of overfeeding were similar in the whole cohort (n = 40) and those that had muscle tissue analyses performed (n = 19) (data not shown).

Table 1 – Anthropometric and metabolic effects of overfeeding.

	Baseline	Day 3	Day 28
Weight, kg	75.3 ± 1.9	75.9 ± 1.9**	78.1 ± 1.9**
BMI, kg/m^2	25.6 ± 0.6	25.8 ± 3.6**	26.6 ± 3.6**
Total body fat, %	34 ± 1	NA	35 ± 1**
Abdominal subcutaneous fat, cm^2	256 ± 17	NA	276 ± 17**
Abdominal visceral fat, cm^2	77 ± 8	NA	86 ± 8**
Fasting glucose, mmol/L	4.5 ± 0.06	4.7 ± 0.06**	4.6 ± 0.05*
Fasting insulin, pmol/L	65.5 ± 3.3	80.3 ± 4.5*	77.2 ± 3.6**
HOMA-IR	1.8 ± 0.1	2.3 ± 0.1**	2.2 ± 0.1**
GIR, μmol/kg FFM/min	54.8 ± 2.8	NA	50.3 ± 2.5*

Data are presented as mean ± SEM. Difference from baseline *P < 0.05, **P < 0.001, NA = not assessed at day 3. Insulin data were log10-transformed prior to statistical analysis. *Abbreviations*: BMI, body mass index; NEFA, non-esterified fatty acids; HOMA-IR, homeostasis model assessment of insulin resistance; GIR, glucose infusion rate; FFM, fat free mass.

3.2. *Changes in Muscle ECM After Overfeeding*

Overfeeding for 28 days resulted in increased COL1 (P < 0.001), COL3 (P = 0.003) and MMP2 (P = 0.05) muscle gene expression, with no significant changes in MMP9, TIMP1, CD68 and integrin α2β1 gene expression (Fig. 1). We also found increases in COL1 and MMP2 between days 3 and 28, with no significant changes in ECM gene expression between baseline and day 3. There were no correlations between changes in ECM gene expression and anthropometric and metabolic changes (day 0–day 28).

3.3. *Microarray Pathway Analyses*

Gene set tests (GSTs) are used to determine if biological pathways or gene sets are differentially up or down-regulated in a dataset. This is based on the assumption that groups of genes in cells often coordinate to make a substantial change instead of large single gene changes, especially in complex metabolic scenarios like pathways involved in obesity, insulin resistance and type 2 diabetes. To determine which gene sets changed in response to overfeeding we compared (1) 0 day *vs* 28 days for all subjects (paired), (2) 28 days only for those who maintained GIR *vs* those who did not (cross-sectional) and 3) 0 day only for those who maintained GIR *vs* those who did not (cross-sectional), considered as the baseline gene set level. We selected gene set changes altered in comparisons (1) + (2) but not in (3) as *bona fide* regulated pathways of interest with 28 pathways of interest identified. Of these 28 pathways of interest, pathways related to ECM receptor interaction, focal adhesion and adherens junction were differentially altered after overfeeding. Other pathways were those involved in amino acid metabolism, carbohydrate metabolism, nucleotide metabolism, signal transduction, transport and catabolism, and the immune system (Fig. 2).

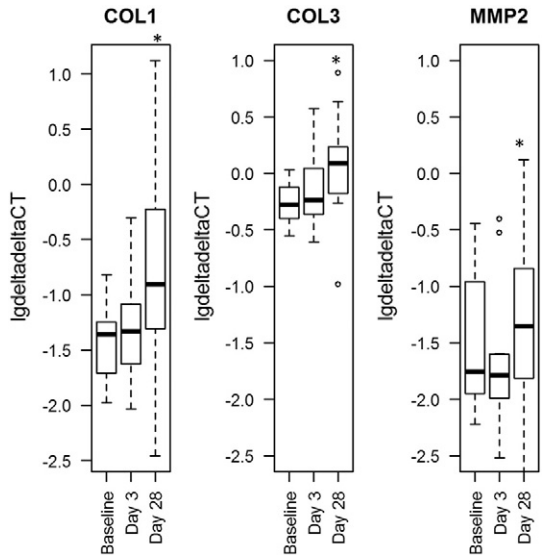

Fig. 1 – Gene expression levels of COL1, COL3 and MMP2 in vastus lateralis at baseline and after 3 and 28 days overfeeding. * p < .05.

4. Discussion

Short-term overfeeding for 28 days resulted in weight gain, increased visceral and total fat deposition and reduced insulin sensitivity along with significantly increased mRNA levels of COL1, COL3 and MMP2 in *vastus lateralis*. Microarray gene set tests also revealed that pathways related to ECM receptor interaction, focal adhesion and adherens junctions were differentially altered after overfeeding. Collagen is the major structural protein in skeletal muscle ECM with COL1 arranged in thick bundles predominantly in the periomysium and providing tensile strength. COL3 is evenly distributed between the endomysium and epimysium as a thin meshwork of fine fibrils, providing plasticity and flexibility [10]. Previous studies have reported significantly increased COL1 and 3 mRNA levels in *vastus lateralis* during experimental insulin resistance produced by lipid infusion [11] and dramatic increases after 10% weight gain induced by overfeeding [7]. COL1 and 3 immunofluorescence was also more abundant in subjects with obesity and/or diabetes, compared to lean individuals [3]. It is not yet known whether muscle accumulation of COL1 and 3 is a pathological response to obesity-related insulin resistance or a normal physiological response to weight gain, although our study also found that MMP2 mRNA levels, a matrix metalloproteinase involved in the breakdown of ECM, is increased after 28 days of overfeeding. Together these findings suggest that active ECM remodeling is occurring in skeletal muscle during obesity-related insulin resistance, rather than ECM accumulation which may reflect more chronic stages of the obesity disease process such that occurs in adipose tissue [2]. Future investigations examining factors that promote or inhibit the synthesis or degradation of the ECM such as matrix metalloproteinase activity and tissue inhibitor matrix metalloproteinases may provide further insight. Together with previous studies [3–5,7], our findings of increased COL1, COL3 and MMP2 mRNA levels in *vastus lateralis* after short-term overfeeding contribute to the larger body of evidence linking skeletal muscle ECM remodeling and accumulation as another sequela of obesity-related insulin resistance. We

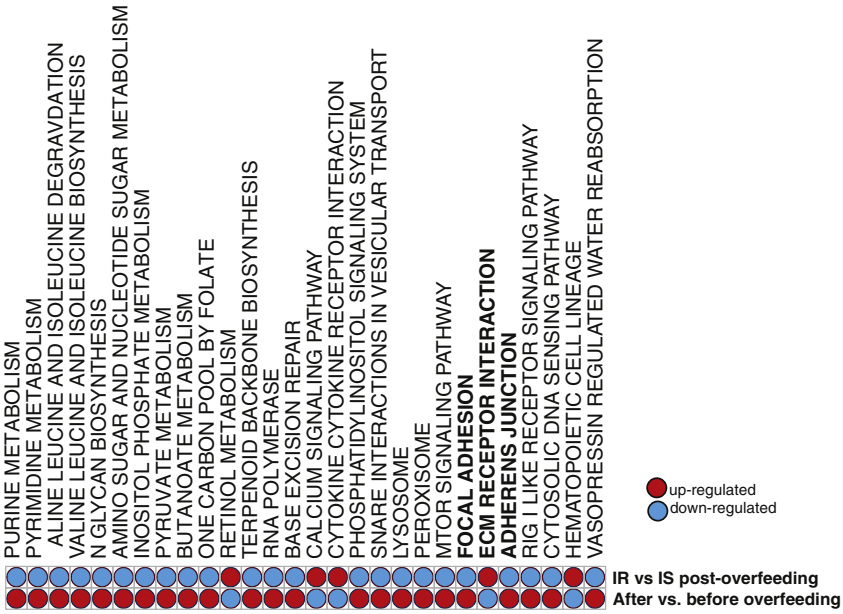

Fig. 2 – Gene set tests showing differentially altered pathways in skeletal muscle between i) individuals at baseline and 28 days after overfeeding and ii) those that had the largest vs. smallest change in glucose disposal rate and had gained at least 2kg after overfeeding. 28 pathways were differentially altered with 3 pathways associated with ECM remodelling (focal adhesion, ECM receptor interaction, adherens junction).

conclude that changes in skeletal muscle ECM remodeling occur early in the obesity disease process with as little as 3% weight gain.

Author Contributions

CST and RC analyzed data and wrote the manuscript. AH and DSB collected data and performed experiments. LKH designed and oversaw the study.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2016.10.009.

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