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Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids?

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Abstract Intra-myocellular triglycerides (IMTG) accumulate in the muscle of obese and endurance-trained (ET) humans and are considered a pathogenic factor in the development of insulin resistance, in the former. We postulate that this paradox may be associated with the peroxidation status of the IMTG. IMTG content was the same in the obese and ET subjects. The lipid peroxidation/IMTG ratio was 4.2-fold higher in the obese subjects. Hence, obesity results in an increased level of IMTG peroxidation while ET has a protective effect on IMTG peroxidation. This suggests a link between the lipid peroxidation/IMTG ratio and insulin resistance.

Key words: Lipid peroxidation; Muscle; Obesity; Endurance training; 4-Hydroxynonenal; Intra-myocellular triglyceride

1. Introduction

Triglycerides accumulate in human skeletal muscle under various metabolic and apparently diametrically opposed conditions. In obesity, the observed accumulation of intra-myocellular triglycerides (IMTG) [1] is hypothesized to be due to a decreased muscle fatty acid β-oxidation activity [2]. In endurance-trained (ET) athletes IMTG accumulation also occurs despite an increased oxidative capacity [3].

In obese or non-obese diabetic subjects, IMTG content is correlated with insulin resistance [3]. Perseghin et al. [4] found in offspring of diabetic patients that IMTG and free fatty acids were the main predictors of whole body insulin resistance. In ET athletes, despite IMTG accumulation, insulin sensitivity was found to be normal. This observation, called the ET athlete paradox, contradicts the observed association between IMTG level and insulin resistance [1,3,4].

Lipid accumulation in steatotic liver is associated with a phenomenon of peroxidation [5] analogous to that observed in the low density lipoproteins (LDL) of the atherosclerotic lesion [6]. It has also been shown, in obese girls, that their LDL are more susceptible to oxidative stress than in normal controls [7]. We hypothesized that IMTG, under certain circumstances, may undergo increased peroxidation. To test this hypothesis, we measured and compared the triglyceride levels and their degree of peroxidation in muscle biopsies from lean subjects before and after 6 weeks of ET and from obese patients.

2. Materials and methods

2.1. Subjects

Five obese male patients and seven lean healthy males (age range and mean ± S.D., 36–62, 34 ± 12 and 27–41, 34 ± 5 years; body mass index, 40 ± 12 and 23 ± 3) participated in this study. The obese patients had a reduced insulin sensitivity as demonstrated by an M value of 8 ± 7 mg/min/kg fat free mass. The M value is an indication of whole body glucose disposal as assessed during a euglycemic hyperinsulinemic clamp. The lean healthy subjects performed 6 weeks of supervised ET and were thereafter referred to as ET subjects. Informed consent was obtained from all subjects and the study, which was approved by the local ethics committee, was carried out in accordance with the principles of the Declaration of Helsinki.

2.2. Muscle sampling, treatment and analysis

Skeletal muscle samples were obtained under local anesthesia from the belly of the vastus lateralis muscle using a percutaneous needle biopsy technique. The muscle biopsies were covered in OCT® medium and immediately placed into isopentane that had cooled to its freezing point in liquid nitrogen.

2.3. Endurance training

The lean controls participated in 6 weeks of ET consisting of three supervised training sessions per week. These included a constant intensity run performed at 60% of $VO_{2,max}$ and two interval sessions performed at 70–80% of $VO_{2,max}$. The interval sessions consisted of six to eight repetitions lasting for 1–3 min and separated by a 1 min recovery.

2.4. IMTG content

Lipid accumulation was determined using an Oil red oil (ORO) stain. In brief, muscle sections were incubated in formalin for 10 min, washed 3 × 30 s in deionized water before staining for 7 min with the ORO solution. After washing again for 3 × 30 s the sections were counterstained with Harris' hematoxylin for 4 min and then rinsed under running tap water for 3 min. The sections were covered with a coverslip and viewed using a Zeiss Axiophot I microscope mounted with an Axiocam color CCD camera. The IMTG were quantified using the Zeiss KS400 V3.0 program. Approximately 375 ± 142 fibers were scanned for each subject.

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Abbreviations: IMTG, intra-myocellular triglycerides; 4-HNE, 4-hydroxynonenal; ET, endurance-trained; ORO, Oil red oil

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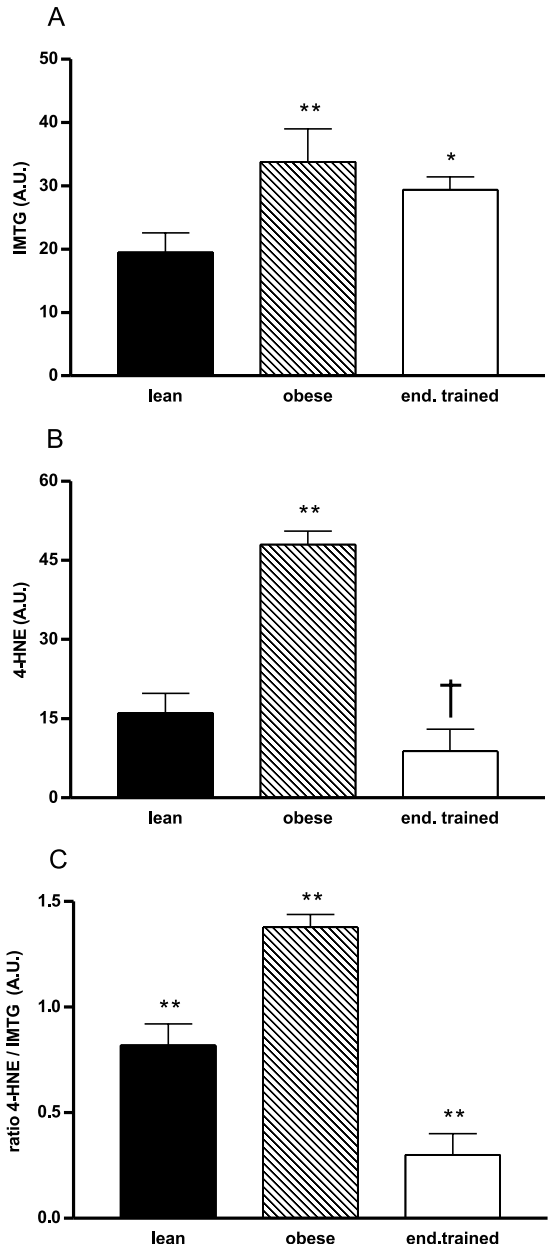

Fig. 1. A: Level of IMTG in muscle biopsies of lean ($n=6$), obese ($n=5$) and ET ($n=6$) subjects; $^{**}P<0.001$ and $^{*}P<001$ vs. lean levels. B: Levels of the lipid peroxidation by-product, anti-4-HNE-lysine in muscle biopsies of lean, obese and ET subjects; $^{**}P<0.001$ vs. lean and ET subjects; $^{\dagger}P<0.05$ vs. lean subjects. C: The relative amount of peroxidized IMTG expressed as the 4-HNE/IMTG ratio in muscle biopsies of lean, obese and ET subjects; $^{**}P<0.001$ for all comparisons. The results are means ± S.E.M.

2.5. Measurement of lipid peroxidation product

4-Hydroxynonenal (4-HNE), naturally produced by non-enzymatic lipid peroxidation of long chain polyunsaturated fatty acids, is a marker of oxidative damage [8]. An immunofluorescence technique, described previously [9], was performed using a mouse monoclonal antibody raised against the 4-HNE-lysine adducts [8] and an Alexa Fluor® 594 goat anti-mouse IgG antibody (Leiden, The Netherlands). The fluorescent adducts were quantified using the Zeiss KS400 V3.0 program [9]. Approximately 423 ± 126 fibers were scanned for each subject.

2.6. Statistics

Analysis of variance was used to determine any differences between the lean, obese and ET groups, followed by a Tukey–Kramer post-hoc test. The significance level was set at $P<0.05$.

3. Results

IMTG contents were, respectively, 1.8- and 1.5-fold higher in obese patients ($P<0.001$) and in ET subjects ($P<0.01$) than in lean controls, while there was no difference in the IMTG contents between the obese and ET groups ($P>0.05$) (Fig. 1A). Lipid peroxidation levels were three-fold higher in the obese patients ($P<0.001$) but were decreased by 45% in ET subjects ($P<0.05$) as compared to lean controls. They were 5.4-fold higher in the obese than in the ET group ($P<0.001$) (Fig. 1B). The relative amounts of peroxidized IMTG, expressed as the 4-HNE/IMTG ratio, were 1.7-fold higher in obese patients ($P<0.001$) and decreased by 63% in the ET subjects ($P<0.001$) as compared to lean controls. This ratio was 4.6-fold higher in the obese than in the ET group ($P<0.001$) (Fig. 1C).

Fig. 2 is a representative immunofluorescence staining of the lipid peroxidation by-product 4-HNE and an ORO staining of IMTG in muscle sections from the obese, lean and ET subjects.

4. Discussion

Our study is the first to show a dramatic difference in the degree of lipid peroxidation, as determined by the quantity of 4-HNE-lysine adducts [8], in the muscle of obese as compared

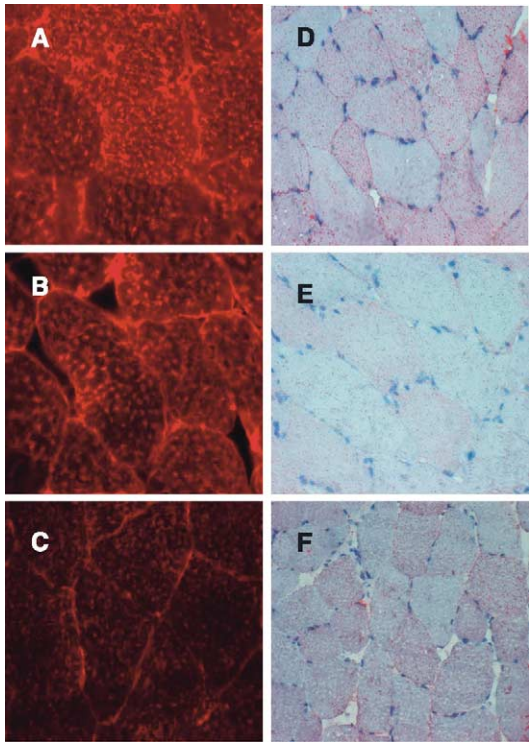

Fig. 2. A representative immunofluorescence staining of the lipid peroxidation by-product 4-HNE, and an ORO staining of IMTG. A–C: 4-HNE in an obese, a lean and an ET muscle section, respectively. D–F: IMTG in an obese, a lean and an ET muscle section, respectively.

to ET subjects. This observation suggests the existence of unknown factors favoring lipid peroxidation in the muscle of obese patients and protecting against it in that of ET subjects. What could these factors be? In obese subjects there is an increase in key indices of lipid peroxidation, such as thiobarbituric acid-reactive species [10], that might participate in the observed increase in IMTG peroxidation. By contrast, in ET athletes, there is an up-regulation of muscle antioxidant enzyme activity [11] which may protect against lipid peroxidation.

The results of the present study suggest a new concept, that of good vs. bad lipids, respectively associated with low and high levels of lipid peroxidation. The IMTG that are stored as an adaptation to ET and are constantly mobilized, as they provide an energy source for ATP production during exercise, are good lipids. In contrast, the lipids stored but not mobilized, as is the case in obese subjects, are bad lipids. These bad lipids may affect insulin sensitivity by the production of lipid peroxidation by-products, such as 4-HNE and/or malondialdehyde. The concept of good vs. bad lipids may explain, in part, the ET athlete paradox [3]. The link between the peroxidation state of IMTG and insulin resistance is still speculative, as is the mechanism that would link the peroxidation state of IMTG and muscle insulin resistance. The bad lipids could affect insulin sensitivity by controlling the level of paracrine factors, such as tumor necrosis factor-α, which are potentially important players in insulin resistance; however, this hypothesis requires further investigation. The concept of good vs. bad lipids should stimulate research to find out if lipid peroxidation affects insulin sensitivity in muscle and, if it does so, by what mechanism(s).

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