Up-Regulation Mttp and Apob Gene Expression in Rat Liver is Related to Post-Lipectomy Hypertriglyceridemia

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Abstract
Background/Aims: The aim of this study was to explain the molecular basis for elevated concentrations of circulating triglycerides (TAGs) after partial surgical removal of adipose tissue (lipectomy) in rats. Methods: The levels of mRNA and protein: a) involved in synthesis of fatty acids and TAGs; b) participating in TAG-rich lipoproteins assembly and secretion; and c) transcription factors essential for maintaining TAG homeostasis were determined by RT-PCR and Western Blot in the livers of control and lipectomized rats. Results: Partial lipectomy was associated with increase: a) in serum and liver concentration of TAGs, and b) in the liver levels of mRNA of microsomal TAG transfer protein (MTP) and apolipoprotein B-100 (ApoB-100). These changes were tightly associated with up-regulation of Hnf1a and Hnf4a gene expression in the liver. Lipectomy was also reflected by a significant increase in the expression of genes encoding: a) fatty acid synthase (FASN), b) glycerol 3-phosphate acyltransferase 1 (GPAT1), diacylglycerol acyltransferases 1 and 2 (DGAT1 and DGAT2), c) spot 14 protein (S14) and SREBP-1 in the liver. Conclusion: Coordinated up-regulation of Mttp, Apob, Hnf1a, Hnf4a, Fasn, Gpam and Dgat (1 and 2) gene expressions may contribute to the increase in circulating and liver concentrations of TAGs after lipectomy in an experimental rat model.

Introduction

Liposuction is one of the most popular esthetic surgeries worldwide, recommended by the American Academy of Cosmetic Surgery [1]. However, health consequences of...
liposuction are still poorly understood. Moreover, the results of previous studies dealing with the problem in question are inconclusive [2-6]. Nevertheless, some authors showed that liposuction is safe and beneficial procedure resulting in an amelioration of insulin resistance and decrease in the markers of vascular inflammation [2-5].

The reports on changes in serum triglyceride (TAG) concentration following lipectomy in humans are also inconclusive. While some authors showed that liposuction exerts no effect on serum TAG concentration in humans [3, 6], others demonstrated that this procedure is reflected by a decrease in this parameter [7]. Furthermore, some studies documented a post-lipectomy increase in serum TAG concentration [8]. Since an increase in serum TAG concentration was shown to be associated with elevated risk of myocardial infarction, ischemic stroke, preterm death [9-11] and acute pancreatitis [12], the post-lipectomy changes in serum TAG concentration require further study.

Recently, Ling et al. [13] reported that lipectomy results in an increase in serum TAG concentration and up-regulation of liver genes encoding proteins involved in lipid metabolism in rats. They suggested that lower serum concentration of adiponectin may contribute to the post-lipectomy hyperlipidemia [13]. Interestingly, elevated level of serum TAGs was also reported in transgenic mice (A-ZIP/F-1 mice) lacking white adipose tissue (WAT) [14]. These results suggest that limited amount of adipose tissue (as is the case after lipectomy) and/or complete lack thereof (as in transgenic mice) may be associated with hypertriglyceridemia.

Theoretically, the post-lipectomy increase in serum TAGs may result from their enhanced liver synthesis, as well as from the production and release of very low density lipoproteins (VLDL). The synthesis and secretion of TAGs in the liver are dependent on the coordinated function of several genes, including two playing a dominant role in these processes. These are Mttp and Apob genes, encoding microsomal triglyceride transfer protein (MTP) and apolipoprotein B-100 (ApoB-100), respectively. MTP and ApoB-100 play a key role in the assembly and secretion of TAG-rich ApoB-containing lipoproteins, such as VLDL [15-22].

Aside from MTP and ApoB-100, also hepatocyte nuclear factor 4 α (HNF4α), being essential for maintaining TAG homeostasis [23-25], and diacylglycerol acyltransferases 1 and 2 (DGAT1 and DGAT2), i.e. enzymes participating in TAG synthesis, are also involved in the regulation of the TAG-rich lipoproteins secretion [26, 27]. Moreover, the liver biosynthesis of TAGs is enhanced due to increased availability of fatty acids synthesized from glucose in hepatocytes [22]. Also the assembly of VLDL was shown to be dependent on both the fatty acid production and the subsequent biosynthesis of TAGs [22].

To explain potential mechanism(s) leading to the post-lipectomy increase in serum TAG concentration, we examined the expression of genes encoding: a) proteins involved in the synthesis of fatty acids (fatty acid synthase, FASN) and TAGs (glycerol 3-phosphate acyltransferase, GPAT1, DGAT1 and DGAT2), b) proteins participating in the TAG-rich lipoproteins assembly and secretion (MTP and ApoB-100), and c) proteins essential for maintaining TAG homeostasis (HNF4 α, HNF1α) in control and lipectomized rats.

Materials and Methods

Animals and surgeries

The rats fed commercial diet, composition of which has been described previously [28], were treated as described recently [29]. Briefly, 12-week-old male Wistar rats were randomly divided into two groups: 1) lipectomized rats (n=10) subjected to resection of epididymal and retroperitoneal WAT, and 2) controls (n=10) that underwent anesthesia and incision of the skin and muscles without the removal of WAT (sham surgery). After 30 days, the lipectomized rats were anesthetized again with subsequent removal of subcutaneous WAT, and the controls were subjected to another sham surgery. Mean weight of WAT removed from the lipectomized rats was 7.7 ± 0.6 g (3.8 ± 0.3 g, 2.0 ± 0.4 g and 1.9 ± 0.4 g for subcutaneous, epididymal and retroperitoneal WAT, respectively). The lipectomy was performed as the two-step procedure in order to reduce perioperative mortality. The surgeries were conducted carefully to avoid bleeding. All the procedures involving animals and their care were approved by the Institutional
Ethics Committee. The rats were anesthetized and killed by decapitation (between 8:00 a.m. and 10:00 a.m.) after 90 days from the first surgery. Blood samples from the carotid artery were collected to the tubes without anticoagulant, centrifuged at 3000 × g for 15 min at 4°C, and the serum was stored at -20°C. The liver fragments were obtained, immediately frozen in liquid nitrogen and stored at -80°C until analysis. Epididymal, retroperitoneal and subcutaneous WAT from the controls, as well as the residual WAT from the lipectomized animals, were removed and weighted.

**RNA isolation and mRNA level determination**

Total cellular RNA was isolated from the frozen liver samples with a commercial RNA isolation kit (Total RNA Mini, A&A biotechnology, Poland). RNA concentration was determined on the basis of absorbance at 260 nm; all the samples showed 260/280 nm absorbance ratio of about 2.0. Prior to the reverse transcription, the RNA samples were treated with RNase-free DNase I (Fermentas, International Inc., Canada). First strand cDNA synthesis and the determination of mRNA levels by RT-PCR were performed as described previously [29], using a Chromo 4 Real-Time Detection System (Bio-Rad Laboratories Inc., USA). The primer sequences used in this study are presented in Table 1. β-actin mRNA was used as an internal standard. Relative quantities of the transcripts were calculated using the 2^ΔΔCT formula [30]. The amplification of specific transcripts was further confirmed on the basis of the melting curve profiles.

**Determination of liver TAGs**

Liver lipids were extracted with Folch method [31] and concentration of TAGs was determined by means of Oil Red O spectroscopic assessment [32] with triolein as a standard.

**Determination of serum TAGs**

Serum TAG concentration was determined using a routine method, at the Central Clinical Laboratory, Medical University of Gdansk.

**Fatty acid synthase activity assay**

0.2 g of liver tissue was homogenized in 1.8 ml of ice-cold buffer (25 mM Tris-HCl pH 7.8, 0.2% Triton X-100). The homogenate was centrifuged at 30 000 × g for 20 min at 4°C. The activity of fatty acid synthase (FASN; EC 2.3.1.85) in the supernatant was measured spectrophotometrically as described elsewhere [33], with a Beckman DU 68 spectrophotometer (Beckman Instruments, Fullerton, USA).

**SDS-PAGE and immunoblotting**

Frozen rat liver was homogenized in 20 mM Tris-HCl buffer (pH 7.8) containing 0.2% Triton X-100 and protease inhibitor cocktail (Sigma, USA), and then centrifuged. Aliquots of the obtained supernatants containing 10 μg of protein were separated by 10% SDS-PAGE and electroblotted to Immuno-Blot™ PVDF Membrane (Bio-Rad Laboratories, Hercules CA, USA). The membrane was blocked by incubation with blocking buffer; and then incubated with rabbit polyclonal anti-HNF4α antibody (NBP1-00876, Novusbio), mouse monoclonal anti-HNF1α antibody (GTX12064, GeneTex), mouse monoclonal anti-MTP antibody (sc-135994, Santa Cruz, CA, USA), rabbit polyclonal anti-ApoB-100 antibody (sc-25542, Santa Cruz, CA, USA), mouse monoclonal anti-FASN antibody (sc-55580, Santa Cruz, CA, USA), and rabbit polyclonal anti-actin antibody (A 5044, Sigma-Aldrich). Secondary HRP-conjugated antibodies were obtained from Sigma Aldrich (A0545, A9044). The reactions were visualized with a SuperSignal West

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**Table 1. Primer sequences used in the study**

| Gene   | Primer sequence (5’-3’) |
|--------|-------------------------|
| Hnf1α  | F: AAGATGACACGAGTACGATGG R: GGTGAGACCGTATGTGCC |
| Hnf4α  | F: AAATGGCAAGTGTTGAACCA R: CAGGTCGCCTCGTGAAGAATC |
| Thsp   | F: GGTTACGGCCTTGTTGGCGG R: AGATCGGGGCGTCGGTGC |
| Sreb1f | F: TCAGTTCCAGCATGGCTACC R: TGGGGAATGTGCTTACGAG |
| Dgat1  | F: TGTTATCTTGTTGGCCTGGC R: GGGTCCTACACCTTCCGGT |
| Dgat2  | F: GAATGGATGGTGTTTCAGCSTAGA R: CAGGTCGAACGGTCCAGCA |
| Fasn   | F: ATGGGAAACGTCGTGCTGCAAT R: TGTTGATATGTTGGATGATA |
| Gpam   | F: TCTGCCGCTCTTGGTTCTGCGG R: GGATGGTTAGGTTGGTGCTGCC |
| Mttp   | F: AAGGCAATATATGAACATCCAGGT R: TGTTATTAACACACGCCACCTGAG |
| ApoB   | F: AGCTGCTGAAGAACGTAGGAAGG R: AATCTGCTGAGGAAAGCCTCTCAG |
| Actb   | F: GAAATCGTGCGTGACATTAAG R: GCTAGAAGCATTTGCGGGTGA |
Pico chemiluminescent substrate (Thermo Fisher Scientific Inc., Rockford, IL, USA). The bands (visible on the film after the chemiluminescent detection) were compared to molecular mass protein markers (SM1811) obtained from Fermentas, visible on the membrane after electroblotting. The film was adjusted to the membrane in such way that the membrane edges were visible on the film.

Statistical analysis

Statistical analysis was conducted using a MS Excel 2010 spreadsheet (Microsoft). All the data were expressed as mean values (± SD) for the controls and lipectomized rats. The significance of intergroup differences in the analyzed parameters was verified with Student t-test. The differences were considered significant at p-value <0.05.

Results

The effects of lipectomy on the mass of subcutaneous (inguinal), retroperitoneal and epididymal WAT were recently reported [29]. Briefly, lipectomy resulted in a complete reduction of subcutaneous adipose tissue and approximately 80% reduction of retroperitoneal and epididymal adipose tissue as compared to the control rats. Consequently, the overall reduction of subcutaneous, retroperitoneal and epididymal adipose tissue mass in the lipectomized rats corresponded to approximately 90%. Mean baseline body weights of the controls and lipectomized rats were essentially similar (312 ± 18 g vs. 315 ± 19 g). Mean final body weights determined at the end of the experiment were 403±21 g and 407±17 g for the controls and lipectomized rats, respectively.

Partial lipectomy was reflected by approximately 2-fold increase in the serum concentration of TAGs (Fig. 1A), as well as by an increase in the liver TAG content (Fig. 1B). Moreover, higher levels of MTP mRNA were also found in the livers of lipectomized rats (Fig. 2A). The different liver levels of MTP mRNA observed in the controls and lipectomized rats were reflected by intergroup differences in the liver levels of MTP protein documented on Western Blot analysis (Fig. 5). Presumably, enhanced expression of Mttp gene contributed to the increase in the serum and liver TAG concentrations in the lipectomized rats (Fig. 1A and 1B). This assumption was supported by a strong positive correlation found between the liver MTP mRNA level and the serum concentration of TAGs (r = 0.67, p<0.05). Partial lipectomy was also associated with approximately 2-fold increase in the liver level of ApoB-100 mRNA (Fig. 2B). Moreover, we found a strong positive correlation between the liver level of ApoB-100 mRNA and the serum concentration of TAGs (r = 0.82, p<0.05). The different liver levels of ApoB-100 mRNA in the controls and lipectomized rats were reflected by intergroup differences in the liver concentrations of Apo-B100 protein documented on Western Blot analysis (Fig. 5).

![Fig. 1. Triglyceride levels in: A) serum and B) liver of the controls and lipectomized rats. Data presented as mean ± SD. * p< 0.05.](image-url)
Partial lipectomy was reflected by a significant increase in the liver HNF1α (Fig. 3A) and HNF4α (Fig. 3B) mRNA levels. Interestingly, the pattern of changes in the liver HNF1α and HNF4α mRNA levels of the controls and lipectomized rats resembled that observed in the case of MTP and ApoB-100 mRNA levels (Fig. 2A and 2B, respectively). The different liver levels of HNF1α and HNF4α mRNA of the controls and lipectomized rats were reflected by intergroup differences in the HNF1α and HNF4α protein levels documented on Western Blot analysis (Fig. 5). Moreover, strong positive correlations were found between the liver levels of MTP mRNA and HNF1α mRNA (r = 0.85, p < 0.05), as well as between the levels of MTP mRNA and HNF4α mRNA (r = 0.83, p < 0.05). We also found positive correlations between the liver levels of ApoB mRNA and HNF1α mRNA (r = 0.81, p < 0.05), as well as between the levels of ApoB mRNA and HNF4α mRNA (r = 0.88; p < 0.05).

Partial lipectomy was associated with a significant increase in the liver activity of FASN (lipectomy: 1.74 ± 0.38; controls: 1.16 ± 0.2 nmol/min/mg protein), as well as by an increase in FASN mRNA (Fig. 4A) and FASN protein levels (Fig. 5). Importantly, the pattern of changes in the liver levels of FASN mRNA of the controls and lipectomized rats resembled that observed in the case of HNF1α mRNA levels. The livers of the lipectomized rats contained more SREBP-1 mRNA than the control livers (Fig. 4B). Moreover, we observed an up-regulation of Thrsp gene (Fig. 4C) in the livers of the lipectomized rats. This gene encodes S14 (spot 14 protein) being critical for the activation of lipogenic enzymes, synthesis of lipids and export of VLDL [34, 35]. Furthermore, lipectomy resulted in an increase in the mRNA levels for glycerol 3-phosphate acyltransferase 1 (GPAT1) (Fig 4D), an enzyme involved in the initial step of the liver biosynthesis of glycerolipids (including TAG) [36], as well as in an increase in the mRNA levels of diacylglycerol acyltransferases 1 and 2 (DGAT1 and DGAT2) (Fig. 4E, 4F), being involved in the final step of TAG biosynthesis in the liver and other tissues [37].
Discussion

To the best of our knowledge, this study was the first to show that the surgical removal of adipose tissue may be associated with coordinated up-regulation of \( \text{Mttp} \) and \( \text{Apob} \) genes in the liver. These changes were associated with the increase in the serum and liver concentrations of TAGs. This suggests that the up-regulation of proteins involved in the assembly and secretion of TAG-rich lipoproteins may contribute to an increase in the serum concentration of TAGs after lipectomy. Taking into account the results of previous studies in which HNF1\( \alpha \) and HNF4\( \alpha \) were shown to be transcriptional activators of \( \text{Mttp} \) and \( \text{Apob} \) genes \([38, 39]\), and our hereby presented findings, we postulate that these hepatocyte nuclear factors (HNFs) might contribute to the hypertriglyceridemia observed in lipectomized rats. This is consistent with the results of a recent study in which lipectomized rats presented with higher concentrations of circulating TAGs and higher liver levels of HNF4\( \alpha \) mRNA than the sham-operated controls \([13]\). Further evidence supporting a role of HNFs in the post-lipectomy increase in serum TAGs comes from a study of \( \text{Mttp} \) and \( \text{Apob} \) expressions and serum TAG concentrations in mice lacking liver \( \text{Hnf4a} \); these animals presented with significantly lower serum concentrations of TAGs \([40]\). Furthermore, Yin et al. \([41]\) observed a significant decrease in the liver expressions of \( \text{Mttp} \) and \( \text{Apob} \) as well as an impairment of

![Fig. 4. The mean A) fatty acid synthase (FASN), B) sterol regulatory element binding protein 1 (SREBP-1), C) Spot 14 protein (S14), D) glycerol 3-phosphate acyltransferase 1 (GPAT1), E) diacylglycerol acyltransferases 1 (DGAT1) and F) diacylglycerol acyltransferases 2 (DGAT2) relative mRNA levels in the livers of the controls and lipectomized rats. Data presented as mean ± SD. * \( p < 0.05 \).](image-url)
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Fig. 5. Western Blot analysis of liver MTP, HNF1, HNF4, FASN, ApoB-100 standardized against actin in the controls and lipectomized rats.

Fig. 6. Proposed mechanism showing how HNFs may affect circulating TAG concentrations in lipectomy rats. Lipectomy is associated with increase in Hnf4a and Hnf1α genes expression and consequently with increase in HNF1α and HNF4α protein levels. In turn, HNF1α upregulates Mttp and ApoB genes expression, products of which increase the VLDL production and secretion. HNF1α increases SREBP-1 production, which upregulates genes encoding enzymes involved in fatty acids and TAG synthesis. Moreover, HNF1α might directly increase the expression of Fasn, and subsequently FASN level. For details see Discussion.

VLDL secretion in Hnf4a deficient mice. Taken together, the results of previous studies and our findings suggest that HNFs may significantly affect metabolism of lipids in rats subjected to surgical removal of adipose tissue. However, the reasons behind the increase in the liver expressions of Hnf1α and Hnf4a after surgical removal of adipose tissue are still unclear. The expression of Hnf4a was postulated to be down-regulated by cytokines [42], and removal of adipose tissue was shown to be reflected by a decrease in the circulating levels of pro-inflammatory cytokines [43, 44]. Thus, it can be hypothesized that surgical removal of adipose tissue in rats may result in a decrease in cytokine levels, which in turn enhances the expression of Hnf4α gene.

It has been suggested that the expression Hnf1α is regulated by HNF4α which binds to a specific nucleotide sequence present in the promoter of this gene [45]. Thus, HNF4α may enhance the expression of Hnf1α in the livers of lipectomized rats and play crucial role as a regulator of Mttp and Apob genes. While several authors postulated that HNF4α is an upstream transcription factor of HNF1α, some researchers suggest that it is rather HNF1α which regulates the expression of Hnf4a [45, 46]. Nevertheless, the reciprocal relationship between HNF1α and HNF4α may play a role in the control of genes encoding proteins involved in the assembly and secretion of VLDL.
As mentioned above, aside from MTP and ApoB-100, also DGAT1 [26] and DGAT2 [27] are involved in the regulation of TAG-rich lipoprotein secretion. Moreover, DGAT1 was shown to contribute to the effect of HNF4α on the liver secretion of TAG-rich protein in human hepatoma cells [24]. Our findings suggest that both DGAT1 and DGAT2 were up-regulated in the livers of lipectomized rats (Fig. 4). Moreover, the overexpression of DGAT1 and DGAT2 turned out to be associated with the increase in the liver levels of HNFs in our lipectomized rats (Fig. 3 and 4). These observations are consistent with the results of previous studies dealing with the problem in question [42]. Therefore, the up-regulation of HNFs likely results in an up-regulation of several other proteins, such as MTP, ApoB-100 and DGAT (1 and 2) which play a crucial role in the assembly and secretion of VLDL, thus leading to hypertriglyceridemia in lipectomized rats.

Liver biosynthesis of TAGs is enhanced due to increased availability of fatty acids synthesized from glucose in hepatocytes [47]. Also the assembly of VLDL and resultant production of TAGs are known to be fatty acid-dependent [22]. Previous studies showed that fatty acid synthase (FASN), an enzyme involved in the synthesis of fatty acids being essential for TAG production, also is a downstream target for HNF1α [48]. Thus, the up-regulation of Hnf1α is likely associated with the enhancement of Fasn expression in the livers of lipectomized rats. The data presented on Figure 4 suggests that this was the case in our lipectomized animals. Moreover, HNF1α is also involved in the regulation of Srebf gene [48], and the product of this gene (SREBP-1) is a key regulator of lipogenesis [49]. Therefore, the up-regulation of Hnf1α is likely associated with the enhancement of Srebf gene expression in the livers of lipectomized rats. This may explain why our lipectomized rats presented with higher liver levels of SREBP-1 mRNA than the controls (Fig. 4). To summarize, up-regulation of both Hnf and Srebf may contribute to the enhancement of Fasn expression and resultant increase in the lipogenic activity.

Based on the data reported previously, indicating that Mttp, ApoB, Fasn and Srebf-1 are target genes for HNFs [38, 39, 48] and the results presented in this paper, we propose a mechanistic scheme showing how HNFs may affect circulating TAG concentrations in lipectomy rats (Fig. 6). For reasons which are not entirely clear, but could be related to lipectomy, the increase of Hnf4α and Hnf1α genes expression, and subsequently the HNF1α and HNF4α accumulation occur. In turn, HNF1α through binding to mttp and apoB promoters could play a crucial role in controlling mttp and apoB genes expression, products of which govern the VLDL production and secretion. HNF1α through binding to Srebf-1 promoter increases SREBP-1 production, which regulates genes encoding enzymes involved in fatty acids and TAG synthesis. Moreover, HNF1α through binding to Fasn promoter may directly (not involving SREBP-1) increase FASN level. Overall, hypertriglyceridemia is likely accompanied by overproduction of proteins involved in: a) fatty acid and TAG synthesis, and b) assembly and secretion of VLDL. Since HNF1α is an upstream transcription factor for proteins involved in fatty acid and TAG synthesis and proteins participating in assembly and secretion of VLDL, it could be considered as a master regulator of TAG synthesis, and subsequently circulating TAG concentrations in lipectomy rats.

In view of the important role of HNF1α and HNF4α in the lipid metabolism and maintenance of serum TAG concentration in lipectomized rats, one may ask how these findings translate onto humans subjected to liposuction. Taking into account the results of previous studies, namely the fact that: a) serum TAG concentrations were shown to be significantly reduced in HNF4α mutation carriers (HNF4α+ patients with maturity-onset diabetes of the young, MODY1) [50], and b) a significant association was observed between the liver HNF4 and DGAT1 mRNA levels, as well as between DGAT1 mRNA and plasma concentrations of TAGs in humans [42], we hypothesize that similar to the lipectomized rats, patients after liposuction may present with increased liver levels of HNF1α and HNF4α, and resultant enhancement of Mttp and Apob expressions. Ybarra et al. [51] showed that liposuction of subcutaneous abdominal fat in overweight subjects results in an increase in circulating ApoB-100. This observation is in line with our hereby presented findings. However, contrary to other studies [8], these authors [51] observed a post-liposuction
decrease in the serum concentrations of TAGs. Thus, it would be inappropriate to speculate if the changes taking place in humans subjected to liposuction are similar to those observed in our partially lipectomized rats.

In conclusion, our study showed that partial surgical removal of WAT in rats is associated with the coordinated up-regulation of Hnf1a, Hnf4a and genes encoding proteins involved in the synthesis, assembly and secretion of TAG-rich proteins. Consequently, the post-lipectomy increase in serum TAG concentration may result from enhanced synthesis and secretion of TAGs in the liver. Although our findings provide a new insight into post-lipectomy metabolism of lipids, further studies are necessary to determine an association between hypertriglyceridemia and up-regulation of Mttp, Apob, Hnf1a and Hnf4a in humans subjected to liposuction.

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Disclosure Statement

We have no conflict of interest to disclose.

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