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Omentin Increases Glucose Uptake, but Not Insulin Sensitivity in Human Myotubes Dependent on Extracellular Lactotransferrin

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Keywords
Adipokine · Omentin · Skeletal muscle · Diabetes · Lactotransferrin

Abstract

Introduction: Omentin (intelectin-1) is an adipokine produced by the stromal vascular fraction of visceral adipose tissue and has been positively associated with insulin sensitivity. The underlying mechanism of action, however, is largely unknown. It has been described that omentin may increase insulin sensitivity and glucose uptake of adipocytes, but effects on other insulin-sensitive tissues such as skeletal muscle are unexplored. We therefore investigated effects of omentin on insulin sensitivity and metabolism of primary human myotubes. ***Methods:*** Primary human myotubes were treated with 0.5 or 2 µg/mL omentin and subsequently protein detection, glucose uptake assay, lactate assay, and lipidomics analysis were performed. ***Results:*** Omentin did not affect skeletal muscle insulin signaling, as assessed by basal and insulin-stimulated phosphorylation of IRS1 and AKT. Omentin increased basal, but not insulin-stimulated glucose uptake. While increased glycolytic activity was confirmed by elevated lactate release after omentin treatment, effects on cellular lipid composition were limited to an increase in total triacylglycerol concentration. Increased glucose uptake by omentin was counteracted by addition of extracellular lactotransferrin, which can bind to omentin. ***Conclusions:*** Overall, increased basal glucose uptake in skeletal muscle cells suggests differential effects of omentin on insulin-sensitive tissues. Moreover, an involvement of lactotransferrin in omentin's mechanism of action may partially explain contradictory results of epidemiological studies on the role of omentin in different diseases.

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Introduction

Omentin (intelectin-1) is an adipokine secreted by the stromal vascular fraction of visceral adipose tissue and is also expressed in the intestine, heart, and lung [1]. Circulating omentin levels are negatively associated with obesity, blood pressure, and $HbA1_c$ levels, but positively with HDL cholesterol, insulin sensitivity and adiponectin levels [2–4], suggesting a protective effect of omentin in the context of diabetes. In contrast, some prospective studies showed a positive association between circulating omentin levels and a higher risk of type 2 diabetes [5, 6]. The reasons underlying these discrepancies remain elusive, also because a receptor for omentin is undiscovered and knowledge on the mechanism of action is limited.

It has been demonstrated that omentin increased insulin-induced glucose uptake of human adipocytes, potentially mediated by increased phosphorylation of AKT [1]. In human macrophages and cancer cell lines, omentin also induced increased phosphorylation of AKT, but decreased phosphorylation of NFκB [7, 8]. In contrast, pro-inflammatory effects of omentin on human adipocytes, mediated by phosphorylation of ERK and NFκB, have been described [9, 10], suggesting that inflammatory effects of omentin may be cell type-specific.

Although increased insulin sensitivity of adipocytes in response to omentin [1] is in line with epidemiologic studies indicating associations between omentin and systemic insulin sensitivity [4], effects of omentin on other insulin-sensitive tissues such as skeletal muscle may also contribute, but are unexplored. We hypothesized that omentin regulates insulin signaling and metabolism of skeletal muscle and therefore investigated the effect of recombinant omentin on human myotubes.

Methods

Cell Culture

Human skeletal muscle myoblasts from 7 healthy donors (2 females, 5 males, aged 16–32 years, BMI 18.9–32.5 kg/m^2; CC-2580 Lonza, Basel, Switzerland) were cultured in growth medium (F12, αMEM, $NaHCO_3$, pH 7.35; ThermoFisher Scientific, Waltham, MA, US) with 5% fetal calf serum, 50 µg/mL bovine fetuin, 10 ng/mL recombinant human epidermal growth factor, 1 ng/mL recombinant human basic fibroblast growth factor, 10 µg/mL recombinant human insulin, 0.4 µg/mL dexamethasone (all in Supplement Pack for skeletal muscle cells, Promocell, Heidelberg, Germany) and 0.2% antibiotic/anti-mycotic-mix (ThermoFisher Scientific) for 3–4 days. Afterward, they were differentiated in medium (αMEM, $NaHCO_3$, pH 7.35, ThermoFisher Scientific, containing 5.56 mM glucose), containing 2% horse serum and 0.2% antibiotic/antimycotic-mix into myotubes.

After differentiation, cells were washed twice with starvation medium (αMEM, $NaHCO_3$, pH 7.35, 0.2% antibiotic/antimycotic-mix) and incubated for 4 h before recombinant human omentin (derived from HEK293 cells, <0.1 EU/µg endotoxin content, Enzo Life Sciences, Farmingdale, NY, USA) was added at the indicated concentrations. Omentin was reconstituted with addition of BSA according to manufacturer's instructions and cells treated with a corresponding concentration of BSA (Merck, Darmstadt, Germany) served as a control. If indicated, lactotransferrin from human milk (Merck) was added at the indicated concentration together with omentin. In order to assess insulin signaling, insulin (from porcine pancreas, Merck) was added for 10 min after stimulation with omentin for 6 or 24 h.

Protein Detection

Proteins were extracted from cells using RIPA buffer containing 50 mmol/L Tris-HCl (pH 8.0) (Roth, Karsruhe, Germany), 150 mmol/L NaCl (Roth), 1% NP-40 (Abcam, Cambridge, UK), 0.5% sodium deoxycholate (Roth), 0.1% sodium dodecyl sulfate (Merck), 10% PhosSTOP Phosphatase Inhibitor Cocktail (Roche, Basel, Switzerland), and 10% cOmplete Mini Protease Inhibitor Cocktail (Roche). After sonication lysates were centrifuged (10 min, 9,000 *g*) and clean supernatants were used for subsequent protein analysis. Protein content of lysates was quantified using a bicinchoninic acid protein assay (ThermoFisher Scientific) according to the manufacturer's instructions. Protein abundance was determined by Simple Western size-based assays using 12–230 kDa separation modules (Bio-Techne, Minneapolis, MN, USA) according to the manufacturer's instructions. Antibodies against the following targets were used: AKT (#9272), phospho-AKT (Ser473, #9271), phospho-AKT (Thr308, #4056), IRS1 (#3407) and phospho-IRS1 (Ser1101, #2385), GLUT1 (#12939), GLUT4 (#2213), tubulin (#3873) (all from Cell Signaling, Danvers, MA, USA), and GLUT3 (#VPA00651, Bio-Rad, Hercules, CA, USA).

Glucose Uptake Assay

Cells were cultured in 96-well plates in duplicate or quadruplicate and treated as described above. Glucose uptake was determined using the Glucose Up-take-Glo™ Assay (Promega, Madison, WI, USA). Briefly, cells were stimulated with 0.1 µM insulin for 1 h at 37°C and 5% CO_2, washed once with PBS and incubated with 1 mM 2-deoxyglucose in PBS for 10 min at room temperature

before stop and neutralization buffer were added. Afterward, 2-deoxyglucose-6-phosphate detection reagent was added and luminescence recorded after incubation for 1 h at room temperature. Wells with cells, but incubated without 2-deoxyglucose served as background control wells. Luminescence from background control wells was subtracted from other data.

Lactate Assay

Lactate concentrations in cell culture supernatants (duplicates) were determined with an enzymatic assay, in which lactate was oxidized and the resulting H_2O_2 was coupled to the conversion of Amplex Red to fluorescent resorufin by horseradish peroxidase. Briefly, either lactate (Merck) standard or diluted samples were incubated with a reaction mix containing horseradish peroxidase (Merck), lactate oxidase (Merck) and Amplex™ Red Reagent (Life Technologies) for 20 min. Subsequently, fluorescence was detected with a plate reader (Biotek).

RNA Isolation and RT-PCR

Samples were resuspended and stored in QIAzol lysis reagent at −80°C until isolation of RNA was performed with the miRNeasy Mini Kit with additional on-column DNA-digestion using the RNase-free DNase Set according to manufacturer's instructions (Qiagen, Hilden, Germany). RNA was transcribed into complementary DNA using the Quantitect Reverse Transcription Kit (Qiagen). Power SYBR Green PCR Master Mix (Applied Biosystems, Waltham, Massachussetts, USA) was used for RT-PCR in a QuantStudio™ 7 Flex Real Time 384-well PCR System (Applied Biosystems). Expression data were normalized to the housekeeping gene B2M. The following primer sequences were used: B2M: fw ATGAGTATGCCTGCCGTG TG, rv CCAAATGCGGCATCTTCAAAC; SLC2A1: fw TTGGCTCCGGTATCGTCAAC, rv GGCCACGATGCT CAGATAGG; SLC2A3: fw TTTGAAGGTTTTGTTGGC TGAA, rv GGCAAATATCAGAGCTGGGGT; SLC2A4: fw TCTCCAACTGGACGAGCAAC, rv CAGCAGGAG GACCGCAAATA.

Lipidomics

Cell samples were homogenized in 80% MeOH with 320 mg glass beads (0.5 mm, VK-05, PeqLab) using a PeqLab Precellys24 homogenizer. Samples were cooled to 0–3°C and homogenized twice at 5,500 rpm for 25 s with 5 s breaks in-between. The homogenate was further used for three purposes. First, 200 µL of the homogenate was extracted with methyl-tert-butyl ether according to the protocol published by Shashikadze et al. [11]. Second, 35 µL of each sample homogenate was pooled to a total volume of 700 µL and 200 µL were extracted in triplicates in the same way as the individual study samples for quality control (QC) purposes. Finally, the cell count was estimated using DNA levels based on fluorescence labeling with the Hoechst dye (final concentration: 20 µg/mL in PBS) as described by Muschet et al. [12].

Lipids were quantified using the DMS-SLA shotgun lipidomics assay as described in [11] with the difference that we used DMS-SLA software version 1.3key-V3Notably, we used the Lipidyzer Standard Kit (Sciex 5040156), PG Internal Standard Mixture – UltimateSPLASH™ (Avanti 330827), PI Internal Standard Mixture – UltimateSPLASH™ (Avanti 330830), PS Internal Standard Mixture – UltimateSPLASH™ (Avanti 330828), and 15:0-18:1-d7-PA (Avanti 791642) as internal standards for quantification. Tuning of the DMS-cell was performed with the EquiSPLASH™ LIPIDOMIX® (Avanti, 330731-1EA). The system suitability was evaluated with the Lipidyzer System Suitability Kit (Sciex 5040407), and a PG/PI/PS mix which was prepared from 17:0-18:1 PI-d5, 17:0-18:1 PG-d5 to 17:0-18:1 PS-d5 (Avanti 850111L-500ug, 858133L-1mg, 858151L-1mg).

Lipidomics Data Processing

The shotgun lipidomics raw data set contained 1,082 individual lipid species. Data were subsequently preprocessed using R (version 4.2.1). To assure high data quality, a multistep procedure was applied. In the first step of this QC procedure, lipids with missing values in more than 35% in the pool samples were discarded from the data set (n = 182). In the second step, the group-specific missingness was evaluated i.e., whether a specific lipid is observed in only one of the biological groups. Lipids exhibiting a groupwise missingness of more than 50% in all groups were discarded from the data set (n = 3). Next, lipids with a coefficient of variation >25%, determined by the QC-pool samples, were removed from the data set (n = 149). The last QC step comprised the calculation of the dispersion ratio (D-ratio) for each lipid [13]. We used a D-ratio threshold of 50%, as this implies that the technical variance is higher than the biological variance (n = 60 lipids were removed). After QC, 688 lipid species remained in the cell homogenate data set, which contained 460 missing values (equivalent to 3.3% of the data set). Missing values were imputed using the k-nearest-neighbor obs-sel approach with k = 5 nearest-neighbors [14].

Proteomics

Protein abundance of lactotransferrin in supernatants and abundance of LDL receptor related protein 1 (LRP1) in cell lysates was assessed in human myotubes cultured as described above. LC-MS/MS analysis was performed as published previously [15].

Proteomics Data Processing

Acquired raw data were analyzed in the Proteome Discoverer 2.4 SP1 software (ThermoFisher Scientific; version 2.4.1.15) for peptide and protein identification via a database search (Sequest HT search engine) against the SwissProt Human database (Release 2020_02, 20432 sequences), considering full tryptic specificity, allowing for up to one missed tryptic cleavage site, precursor mass tolerance 10 ppm, fragment mass tolerance 0.02 Da. Carbamidomethylation of cysteine was set as a static modification. Dynamic modifications included deamidation of asparagine and glutamine, oxidation of methionine, and a combination of methionine loss with acetylation on protein N-terminus. The Percolator algorithm [16] was used for validating peptide spectrum matches and peptides. Only top-scoring identifications for each spectrum were accepted, additionally satisfying a false discovery rate <1% (high confidence). The final list of proteins satisfying the strict parsimony principle included only protein groups passing an additional protein confidence false discovery rate <5% (target/decoy concatenated search validation).

Quantification of proteins, after precursor recalibration, was based on intensity values (at RT apex) for all unique peptides per protein. Peptide abundance values were normalized on total peptide amount. The protein abundances were calculated summing the abundance values for admissible peptides. The final protein ratio was calculated using median abundance values of five biological replicates each.

Statistical Analysis

Comparisons of two conditions were assessed with paired *t* tests. Otherwise repeated measures one-way ANOVA with post hoc Dunnett's test compared to the control or repeated measures two-way ANOVA with post hoc Sidak's test was used. Statistical analysis was performed with GraphPad Prism 9. Volcano plots were generated with R (version 4.2.1).

Results

To investigate the impact of alterations in omentin concentrations on skeletal muscle, we exposed primary human skeletal myotubes for 6 h or 24 h to recombinant human omentin in concentrations resembling average (0.5 μg/mL) and maximal (2 μg/mL) plasma concentrations in a population-based cohort [4]. We did not detect effects of omentin on major targets in the insulin signaling pathway after 6 h and 24 h, including activating phosphorylation of AKT (Ser473, Thr308) and inhibiting phosphorylation of IRS1 (Ser1101) in the basal state (Fig. 1). We also did not detect any effects of omentin on the insulin-induced changes in phosphorylation of AKT (Ser473, Thr308) and IRS1 (Ser1101) (Fig. 1).

Interestingly, 2 μg/mL omentin increased basal glucose uptake after 6 h by 64% (Fig. 2a) and after 24 h by 56% (Fig. 2b). Consistent with a lack of effects on insulin signaling, we did not detect differences in insulin-stimulated glucose uptake after treatment with omentin (Fig. 2c, d). In addition to increased glucose uptake, we observed increased extracellular lactate release after 24 h treatment with 2 μg/mL omentin (Fig. 2e). We, however, did not detect effects of omentin on expression of the glucose transporters GLUT1, GLUT3, and GLUT4 (online suppl. Fig. 1; for all online suppl. material, see https://doi.org/10.1159/000541915). Next to effects on glycolytic metabolism, omentin may also act on other metabolic pathways, including lipid metabolism, as suggested by studies indicating positive associations of omentin with HDL [3, 4] and negative associations with triglycerides [4]. Therefore, we analyzed intracellular lipid levels 24 h after treatment of cells with omentin. Omentin did not affect most lipid classes, such as ceramides, phosphatidylcholines, and sphingomyelins, but increased intracellular concentrations of 173 triacylglycerol, but only four diacylglycerol species (not significant after adjustment for multiple testing) (Fig. 2f; online suppl. Table 1), which was reflected in an increase of total triacylglycerol concentration (Fig. 2g; online suppl. Table 2).

Currently, a receptor for omentin is unknown. In ovarian cancer cells, however, it has been demonstrated that omentin may alter intracellular metabolism by extracellular interaction with lactotransferrin [17]. It was suggested that by binding to lactotransferrin, omentin may suppress the effect of lactotransferrin on the cells mediated by LRP1 [17] (Fig. 3a). Since lactotransferrin was also secreted by differentiated human skeletal muscle myotubes (Fig. 3b), but not significantly altered by omentin, and LRP1 was present in our cell model (Fig. 3c), we investigated, whether addition of extracellular lactotransferrin may counteract the effect of omentin on glucose uptake of myotubes. Extracellular lactotransferrin significantly attenuated the effect of omentin on basal glucose uptake in the presence of 2 μg/mL omentin by 29% (Fig. 3d).

Discussion

This study demonstrates that omentin does not seem to affect insulin sensitivity, but increases basal glucose uptake and lactate release of human myotubes. In addition,

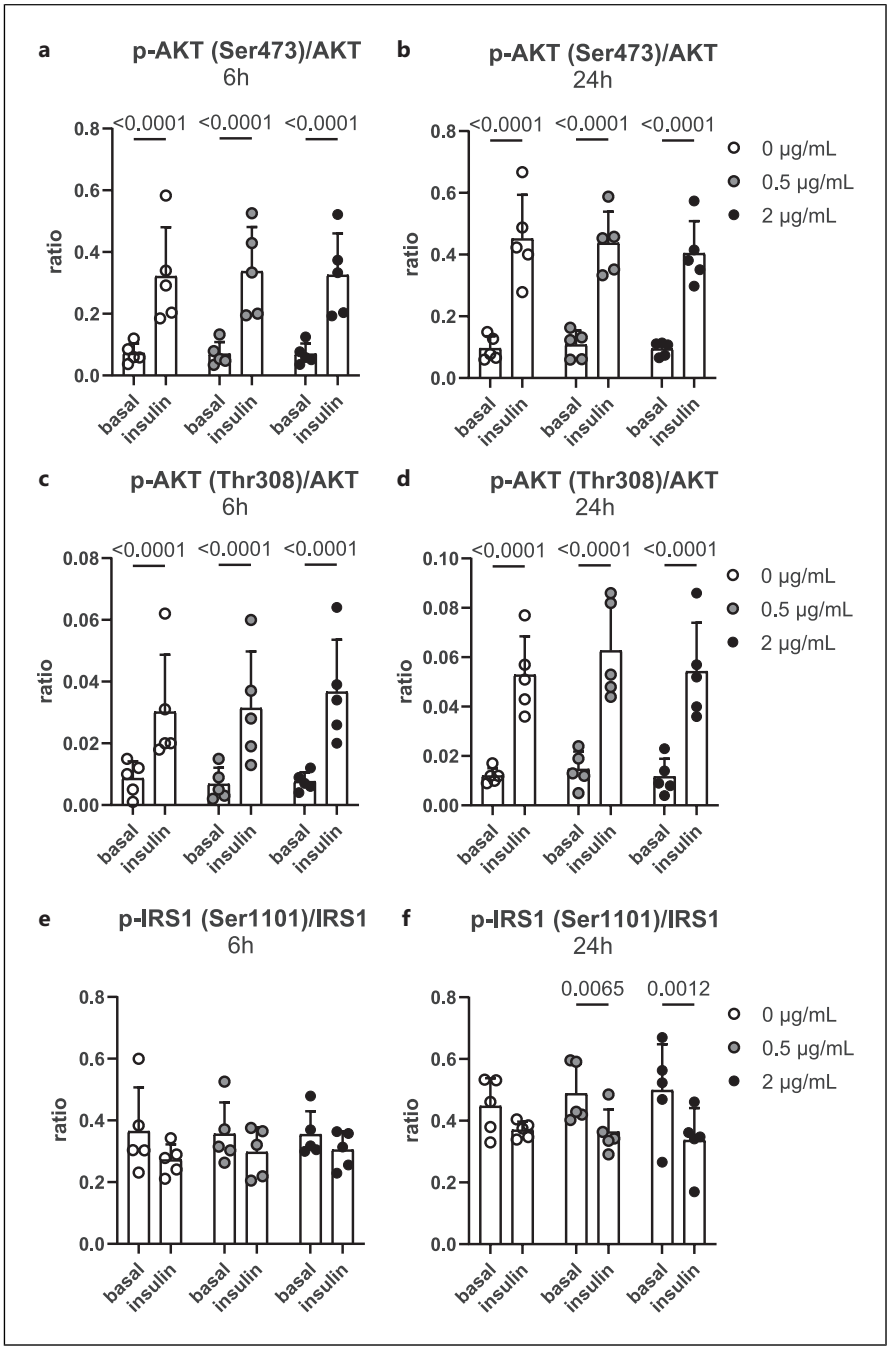

Fig. 1. Omentin has no effect on phosphorylation of AKT and IRS1. **a–f** Phosphorylation of AKT (Ser473) in cells treated for 6 h (**a**) and 24 h (**b**), phosphorylation of AKT (Thr308) in cells treated for 6 h (**c**) and 24 h (**d**) and phosphorylation of IRS1 (Ser1101) in cells treated for 6 h (**e**) and 24 h (**f**) with the indicated concentrations of omentin in the basal state and after stimulation with insulin.

omentin increased intracellular triacylglycerol content. Importantly, we also show that effects of omentin on glucose uptake are partially reversed by lactotransferrin, and thereby highlight a potential mechanism of action for omentin in the context of metabolic diseases.

In contrast to other studies reporting increased phosphorylation of AKT by omentin in adipocytes [1], endothelial cells [18], or macrophages [19], we could not observe any effect of omentin on AKT-phosphorylation in our cell model. On the one hand, this may be due to cell type-specific effects of omentin, which have already been demonstrated for inflammatory pathways [7, 10]. On the other hand, previous studies have investigated short-term effects of omentin (15–120 min), whereas we have investigated more chronic and thus more physiological effects of omentin (6 h and 24 h). Hence, we cannot exclude that omentin

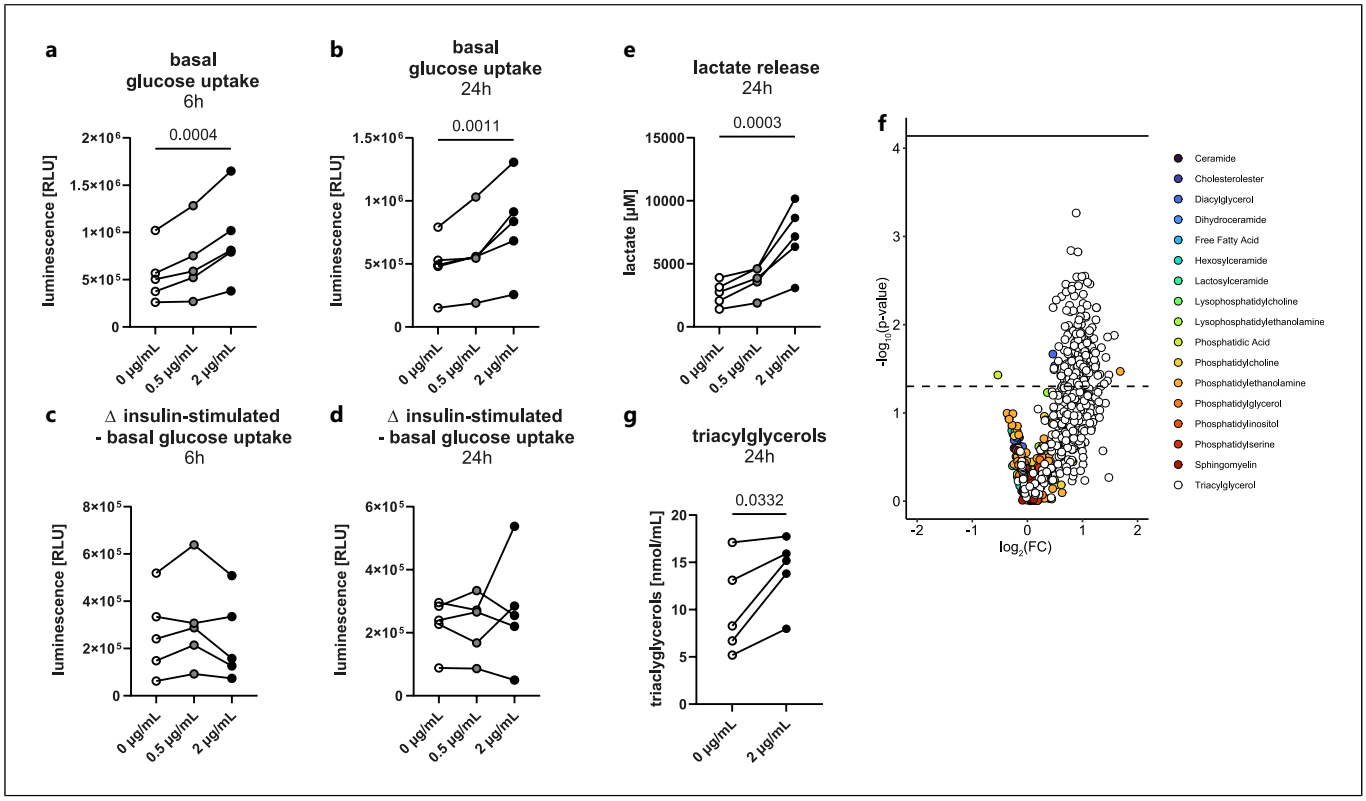

Fig. 2. Omentin increases basal glucose uptake, lactate release and triacylglycerol content. **a**, **b** Basal glucose uptake assessed in cells treated for 6 h (**a**) or 24 h (**b**) with the indicated concentrations of omentin. **c**, **d** Insulin-stimulated glucose uptake (shown as glucose uptake after insulin stimulation – basal glucose uptake) in cells treated for 6 h (**c**) or 24 h (**d**) with the indicated concentrations of omentin. **e** Lactate concentrations measured in supernatants of cells treated for 24 h with the indicated concentrations of omentin. **f** Volcano plot showing differences in concentrations of lipid species assessed by LC-MS/MS in cells treated for 24 h with 2 µg/mL omentin versus control. Dotted line indicates $p = 0.05$, solid line represents the significance threshold adjusted for multiple comparisons ($p = 0.05/688$). **g** Total triacylglycerol concentration assessed by LC-MS/MS in cells treated for 24 h with 2 µg/mL omentin versus control.

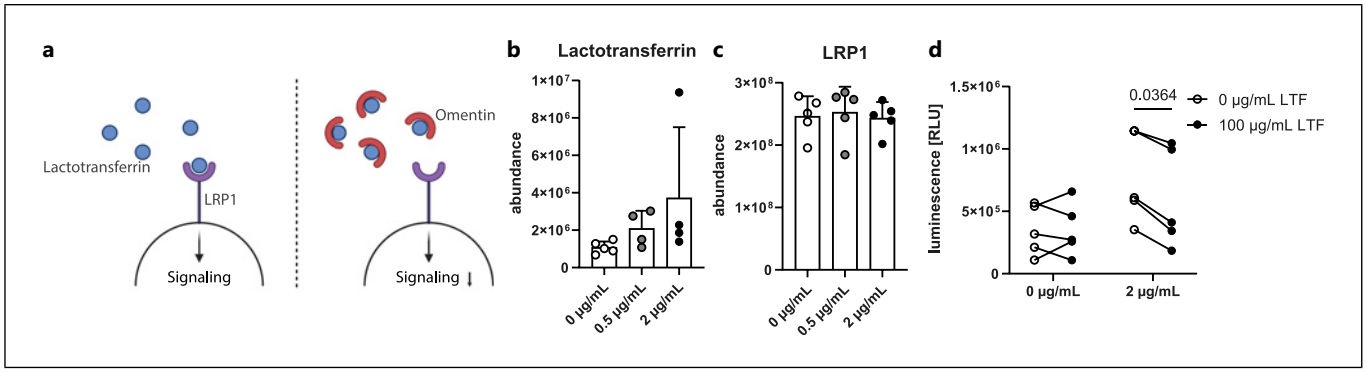

Fig. 3. Lactotransferrin counteracts increased glucose uptake by omentin. **a** Principle of extracellular interaction of omentin with lactotransferrin. Created with BioRender.com. **b**, **c** Protein abundance of lactotransferrin in supernatants of cells (**b**) and protein abundance of the receptor LRP1 in lysates of cells (**c**) treated for 24 h with the indicated concentrations of omentin. **d** Basal glucose uptake in cells treated with 0 µg/mL or 2 µg/mL omentin in the presence of 100 µg/mL lactotransferrin (LTF) versus no lactotransferrin (no LTF).

might induce short-term alterations in AKT or IRS1 phosphorylation in skeletal muscle cells. It is likely that short-term effects of omentin may be more relevant in adipose tissue, the site of omentin production, compared with skeletal muscle, which will be primarily influenced by omentin levels in the circulation. Moreover, long-term exposure to omentin, as applied in our study, may better reflect the alterations in omentin levels occurring in obesity [2] and diabetes [6]. Future studies on the long-term effects of omentin on insulin signaling should demonstrate whether this effect is specific to the skeletal muscle or also occurring in adipose tissue. In endothelial cells, stimulation with omentin increased phosphorylation of AKT up to 2 h, but not after 4 h anymore [18], indicating that the time of exposure to omentin may indeed be relevant for its effect on cellular signaling.

It was previously shown that omentin increased insulin-stimulated, but not basal glucose uptake of adipocytes, potentially mediated by increased phosphorylation of AKT and increased gene expression of GLUT4 [1, 17]. In human myotubes, however, omentin strongly increased basal, but not insulin-stimulated glucose uptake. This observation is in line with a lack of effect of omentin on insulin signaling in myotubes as described above and could be a consequence of cell type-specific effects, as well as different experimental conditions, such as incubation periods. Nevertheless, it has been reported that basal skeletal muscle glucose uptake is reduced in people with type 2 diabetes [20] and may therefore be counteracted by treatment with omentin. Although potential effects of omentin on GLUT4 have been reported in adipocytes [17], we were not able to detect effects of omentin on cellular expression of the glucose transporters GLUT1, GLUT3, and GLUT4 after 24 h in human myotubes. Future studies investigating the effect of omentin on the surface expression and activity of glucose transporters at different time points would therefore be of great interest.

In several studies, positive associations of omentin levels with HDL cholesterol [3, 4], but negative associations with triglyceride levels have been detected [4]. On the cellular level, effects of omentin on lipid composition remain largely unexplored but reduced macrophage lipid load and foam cell formation after treatment with omentin had protective effects against atherosclerosis [7, 21] and suggest a role for omentin in modulating cellular lipid metabolism. Using mass spectrometry, we found no significant increase in specific lipid species and only a minor increase in total triacylglycerol concentration after exposure to omentin. Of note, effects of omentin on cellular lipid metabolism may rely on the individual metabolic state and may therefore be limited in cells from individuals with normal or mild overweight, as present in some of the donors investigated in this study.

Intramuscular triglyceride content associates with insulin resistance [22, 23], but interestingly, it has also been shown that increased intramuscular triglyceride synthesis, e.g., after acute exercise, is positively associated with insulin sensitivity [24] and that synthesis rates of intramuscular triglycerides were positively associated with insulin sensitivity, as well as decreased accumulation of C18:0 ceramides and glucosylceramides [25]. It may therefore be of interest to investigate, whether circulating concentrations of omentin are associated with intramuscular triglyceride content in humans in vivo because this might contribute to the association of circulating omentin levels with insulin sensitivity. Although they have not yet been explored, effects of omentin on cellular lipid metabolism will likely also be relevant in adipocytes and hepatocytes.

In the context of multiple diseases, contradictory associations of omentin have been described. In cardiovascular disease, protective effects of omentin have been reported in experimental studies [7], whereas higher omentin concentrations have been associated with higher risk of stroke in the EPIC cohort [26]. In the context of metabolic disease, higher omentin levels in serum have been associated with higher insulin sensitivity in cross-sectional studies [4, 27], whereas they have been associated with a higher risk of type 2 diabetes in prospective studies [5, 6]. The reasons for these seemingly conflicting results are yet unknown, partially because information on omentin's mechanism of action is limited. Here, we describe that the interaction of lactotransferrin with omentin may not only be relevant in the context of cancer [17], but also in metabolic disease. In our model, lactotransferrin was able to partially block the effect of omentin on glucose uptake. An interaction of omentin and lactotransferrin may affect both the action of omentin and lactotransferrin. Lactotransferrin is an iron-binding glycoprotein of the transferrin family, which has immune-modulating properties [28]. It can be produced by neutrophils, evidenced by correlations between plasma concentrations of lactotransferrin with granulocyte counts and activation [29]. Decreased lactotransferrin levels in individuals with altered glucose tolerance have been suggested to be a marker of neutrophil dysfunction in type 2 diabetes [28]. Whereas one study reported a positive association between circulating lactotransferrin with insulin sensitivity assessed by intravenous glucose tolerance test [28], another study found a positive correlation of lactotransferrin levels with HOMA-IR [30]. Due to the interaction of omentin and lactotransferrin, it will therefore be important to measure both omentin and lactotransferrin in order to clarify their function in obesity and diabetes. An interaction with lactotransferrin has not yet been considered in the various

epidemiologic studies on omentin, but lactotransferrin concentrations may vary in individuals between and/or within different cohorts and may therefore influence the role of omentin in disease. As both omentin [9, 10] and lactotransferrin [28] have been associated with immunomodulating properties, an interaction of the two may have reciprocal effects on their inflammatory action. Investigating the interaction of omentin and lactotransferrin will also be relevant to elucidate the role of omentin in cardiovascular disease because circulating lactotransferrin has, for instance, been shown to predict fatal ischemic heart disease in individuals with type 2 diabetes [31]. Furthermore, it needs to be clarified, whether effects of omentin and lactotransferrin in metabolic disease are mediated via interactions with the receptor LRP1, as was suggested for cancer cells [17].

Although we only studied human primary cells and used omentin concentrations within the physiologic range in human plasma, it is currently unknown whether the local concentrations of omentin in the skeletal muscle environment may differ from those used in our study. Furthermore, we only used cells from donors with normal weight, overweight, and obesity, and therefore, we cannot draw conclusions about the effects of omentin on cells from individuals with diabetes. It is not yet clear, to which extent omentin may have sex-specific effects [6, 32]. In our experiments, we used cells from male as well as female donors and saw consistent effects on, e.g., insulin sensitivity and glucose uptake. Nevertheless, we cannot exclude that omentin has sex-specific effects on the molecular level that we were not able to detect with the limited number of individuals we have investigated.

Conclusion

In conclusion, we show that omentin increases basal glucose uptake of human skeletal muscle myotubes, and that this effect is dependent on extracellular concentrations of lactotransferrin. Hence, future studies on the role of omentin in metabolic disease should further investigate effects of omentin on other insulin-sensitive tissues such as the liver, and consider a role for lactotransferrin.

Statement of Ethics

For this study, only commercially available human myoblasts were used. Based on local and national guidelines further approval of this study by an Ethics Committee was not necessary. Only commercially available cells were used and therefore consent to participate was not required.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions

J.M.R.-R. designed the study together with C.N., C.H., C.W. and M.R. J.M.R.-R., A.Z., C.N., and K.R. performed experiments. J.M.R.-R. analyzed and interpreted data and prepared figures. F.R., M.H., and J.L. were responsible for metabolomics measurements. M.H. analyzed lipidomics data. S.M.H. supervised the proteomics measurements. J.M.R.-R. wrote the original draft of the manuscript. C.H., C.W., and M.R. reviewed and edited the manuscript. All authors were involved in critically revising the article and approved the final version.

Data Availability Statement

All data supporting the findings of this study are available within the paper and its Supplementary Material. Further inquiries can be directed to the corresponding author.

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