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Conditioned media from abdominal and femoral subcutaneous adipose tissue lowers skeletal muscle glucose uptake, not fusion index or myosin thickness

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Short Report

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Abstract

Background:

Abdominal subcutaneous adipose tissue (abSAT), femoral SAT (fmSAT) and visceral adipose tissue (VAT) are all uniquely associated with the development of type 2 diabetes (T2D).

Evidence also shows that skeletal muscle structure changes in individuals with T2D; whether adipose tissue causes these alterations remains unknown. The current study examines how adipose tissue from the three main depots of adipose tissue impacts myogenesis and skeletal muscle glucose uptake.

Methods:

Regional conditioned media were generated with adipose tissue explants taken from eight females living with obesity and T2D. Myocytes were cultured from a healthy female donor and exposed to a 1:1 conditioned-to-differentiation media ratio. Subsequently, myosin heavy chain (MyHC) thickness, fusion index, and glucose uptake were quantified.

Results:

Though conditioned media from VAT had no effect compared with control, conditioned media from abSAT and fmSAT reduced skeletal muscle glucose uptake (~2.1- and ~1.4 fold vs. VAT, respectively; $p<0.01$). No differences in myotube morphology (MyHC thickness, fusion index) were observed across depots, and glucose uptake was independent of muscle structural characteristics and body composition.

Conclusions:

Collectively, these results reveal that abSAT and fmSAT secretions impair glucose uptake in skeletal muscle, without compromising myogenesis.

Introduction

Obesity is the leading risk factor for the development of type 2 diabetes (T2D). With rising rates of obesity in Canada, the prevalence of T2D has risen to approximately 1 in 9 Canadians.[1] The impact of uncontrolled T2D on the individual is significant, potentially leading to diabetic retinopathy, neuropathy, hypertension and impaired cognition - reducing quality of life.

Under insulin-stimulated conditions, skeletal muscle accounts for 80% of glucose uptake.[2] Any disruption in skeletal muscle glucose uptake can significantly perturb whole-body energy homeostasis. Factors originating from adipose tissue in obesity have been shown to reduce insulin-stimulated glucose uptake in muscle, including free fatty acids, adipokines/cytokines, and extracellular vesicles. In line with these findings, individuals with T2D have also been observed to have a decreased ability to grow muscle[3], which coincides with changes in skeletal muscle structure.[4] However, we do not know whether

structural changes in skeletal muscle affect its glucose uptake. Understanding how skeletal muscle structure affects glucose uptake is important for understanding the pathogenesis of T2D.

There are three major depots of adipose tissue in the human body, each with distinct associations with T2D. Studies have shown that individuals with greater central adiposity are at an increased risk for T2D.[5] Visceral adipose tissue (VAT), which accumulates in the abdominal cavity, shows a stronger association with T2D than abdominal subcutaneous adipose tissue (abSAT).[6,7] In contrast, though femoral SAT (fmSAT) remains understudied, fmSAT is believed to be protective against the development of T2D.[8,9] We do not know how adipose tissue from each region impacts skeletal muscle growth and glucose uptake. Our study aimed to examine how abSAT, fmSAT, and VAT from individuals with obesity and T2D affect *in vitro* skeletal muscle growth and glucose uptake. The results of our study will lay the groundwork for future *in vivo* experiments.

Methods

Participants and Study Design

Eight females with a body mass index (BMI) >40 kg/m² and a T2D diagnosis were recruited from the bariatric surgery clinic at Le Centre intégré universitaire de santé et de services sociaux du Nord-de-l'île-de-Montréal (CIUSSS-NIM), Québec. All diabetes medications were excluded, except Metformin and/or Invokana, as these medications have not been shown to affect skeletal muscle glucose metabolism.

Aspirated SAT biopsies were taken approximately 1-3 months before bariatric surgery, prior to presurgical weight loss in an outpatient setting, as previously described.[10] Briefly, a lidocaine/Ringer's lactate solution was superficially injected in a fan-like shape before needle aspiration of adipose tissue using a 9-gauge tri-eye needle. VAT biopsies were excised during surgery from the greater omentum. Previous work has found no difference in the inflammatory characteristics of adipose tissue obtained via surgical excision versus needle aspiration.[11] Satellite cells were isolated from a skeletal muscle biopsy of the vastus lateralis in a healthy female (33y), as previously described.[12] Ethical approval was obtained from the University Human Research Ethics Committee of Concordia University, Comité d'éthique de la recherche du CIUSSS-NIM, Hopital du Sacré-Coeur de Montreal, and Comité central d'éthique de la recherche du ministre de la Santé et des Services sociaux. All participants provided written informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

Body Composition

Body composition was measured using a combined DXA scan (Lunar Prodigy Advance; GE Healthcare, Madison, Wisconsin) and a CT scan (Revolution Evo; GE Medical Systems, Milwaukee, WI, USA) at L2-3. The mass of regional adipose tissue (lower body SAT, upper body SAT, and VAT) were obtained, as previously described.[13,14]

Conditioned Media

Adipose tissue from each region was minced and incubated in media (480mL M199, 250 µL insulin, 125 µL dexamethasone, 5 mL penicillin-streptomycin and 14.6 mL NaHCO3) at a ratio of 1g/3mL at 37 °C in 5% CO2 for 24 hrs. After 24 h, the media was replaced with the same formulation minus insulin and dexamethasone (control media). After an additional 24 h, the resulting conditioned media were aliquoted and stored at -80 °C for later treatment of myocytes. Hormone and protein concentrations in conditioned media were not quantified due to the low volumes of conditioned media required for the co-culture experiments.

MyHC Staining

Satellite cells were seeded onto 24-well plates and cultivated to confluence. For 5 days, cells were treated with a 1:1 conditioned-to-differentiation media (DMEM supplemented with 2% horse serum) ratio. The media was changed every 72h. After the 5 days of myocyte exposure to the conditioned media, myocytes were treated with differentiation media for an additional 48 h. Myocytes were then fixed using 4% paraformaldehyde for 15 min, washed three times with PBS, and permeabilized with 0.3% Triton X-100 in PBS for 12 min at room temperature. Following permeabilization, cells were washed 3 times with PBS, then blocked with 2% BSA for 30 min.

Cells were subsequently incubated with mouse anti-Human MyHC monoclonal antibody (Catalogue # MAB4470, R&D Systems) at a 1:400 dilution in TBST overnight at 4 °C. The following morning, cells were washed 3 times with PBS, then stained with Alexa Fluor 488 Anti-Mouse IgG secondary antibody (Cat#A-11008, Thermo Scientific) at a 1:500 dilution in TBST for 2 hrs at room temperature. Cells were then counterstained with DAPI (Thermo Scientific, Cat#62248) at a 1:1500 PBS ratio.

Fusion index was quantified by counting the number of nuclei in plurinucleated cells as a percentage of the total nuclei. Approximately 1000 nuclei per well were counted in two independent cultures.[15] MyHC thickness was measured with ImageJ software. Each myotube was measured by averaging three thickness points in 10 random fields (x10) from two independent cultures. Figure 1 shows examples of myosin heavy chain staining in healthy skeletal muscle cells exposed to regional adipose tissue conditioned media.

2-NBDG Glucose Uptake Assay

Satellite cells were seeded at a density of $1x10^4$ on a 96-well plate and treated with differentiation media (DMEM supplemented with 2% horse serum) for 5 days. After differentiation, myocytes were treated with conditioned medium at a 1:1 ratio to differentiation medium for 72h. The 2-NBDG Glucose Uptake Assay (Abcam, Cat#ab235976) was followed as previously described.[16] Accordingly, cells were treated with 100nM insulin, and 2-NBDG was added to a final concentration of 60 µM.

Statistics

Statistics were run using IBM SPSS Statistics Software version 28.0.0.0 (Armonk, NY). All data were normally distributed according to the Shapiro-Wilk test. Regional differences were examined using a mixed-model ANOVA with LSD post hoc tests following significant ANOVA results. Associations between MyHC thickness, fusion index and glucose uptake were assessed by Pearson correlation coefficients. Data are presented as mean ± SEM; significance was set at $p<0.05$.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Subject Characteristics

Participant characteristics are described in Table 1. Participants were 41 ± 4 y with a BMI of

Table 1
Participant Characteristics

Characteristic	**OB+T2D (n = 8)**		
abSAT (kg)	36.0	±	2.0
fmSAT (kg)	19.6	±	2.1
VAT (kg)	6.5	±	0.53
Total Adipose Tissue (kg)	61.7	±	9.5
Total Lean Tissue (kg)	56.7	±	4.2
Android:gynoid	0.57	±	0.05
Insulin (pmol/L)	277.8	±	157.2
Glucose (mmol/L)	7.2	±	2.0
HbA1c (%)	7.3	±	2.5
HOMA-IR	14.9	±	10.1

45.9 ± 1.8 kg/m^2 and a HbA1c of 7 ± 1%. Regional adipose tissue mass was greatest ($p < 0.001$) in abSAT, followed by fmSAT and then VAT (36.0 ± 2.0 kg, 19.6 ± 2.1 kg, and 6.5 ± 0.53 kg, respectively).

Effects of Adipose Tissue-derived Conditioned Media on Myotube Morphology and Glucose Uptake

Myocytes treated with conditioned media derived from abSAT and fmSAT exhibited ~ 2.1 and

~ 1.4 times lower 2-NBDG glucose uptake, respectively, compared with myocytes exposed to conditioned media from VAT ($p < 0.001$ and $p < 0.01$, respectively), and the control media ($p < 0.01$ and $p = 0.03$, respectively) (Fig. 2). There were no differences in glucose uptake between myocytes treated with conditioned media from abSAT and fmSAT. As shown in Fig. 2, no differences in glucose uptake in myocytes were detected after exposure to VAT or control media.

In contrast, myotube morphology was not altered by the adipose tissue depot. MyHC thickness and fusion index were similar across all conditioned media treatments (Fig. 3), indicating that differences in glucose uptake occurred independently of myotube differentiation

or hypertrophy. Glucose uptake was not associated with body composition, MyHC thickness or fusion index (data not shown).

Discussion

This present study is the first to examine how all three primary human adipose tissue depots (abSAT, fmSAT and VAT) from females with T2D influence muscle myogenesis and glucose uptake *in vitro*. A key novel finding of our study is that conditioned media derived from abSAT and fmSAT reduced skeletal muscle glucose uptake compared with VAT-conditioned and control media. Importantly, these impairments occurred independently of any alterations in MyHC thickness or fusion index, suggesting that adipose-tissue-mediated reductions in glucose uptake are not driven by overt changes in myotube structure or differentiation. These findings support the concept that, in T2D, skeletal muscle insulin resistance may arise from functional disruptions in glucose handling rather than from structural remodelling alone. Moreover, the observation that fmSAT- conditioned media impaired glucose uptake challenges the traditional view that femoral adiposity is relatively benign and suggests that, in the context of T2D, fmSAT may exert previously underappreciated pathogenic effects. By assessing the effects of regional adipose tissue-conditioned media on myotube morphology and glucose uptake, the work provides an integrated view of how depot-specific adipose tissue may contribute to skeletal muscle metabolic dysfunction in a physiologically relevant human T2D model.

Our observation that adipose tissue impairs glucose sensitivity is consistent with several animal studies that have used various methods to demonstrate that exposure to adipose tissue-conditioned media impairs the insulin signalling pathway in animals.[20–22] When L6 myocytes from rats were co-cultured with murine 3T3-L1 cells, insulin-stimulated glucose uptake was impaired.[21] Furthermore, compared to control adipocytes, co-culture of L6 myocytes with primary rat adipocytes from the streptozotocin-induced diabetes rat model resulted in blunted glucose uptake.[21,22] In line with these findings, we

observe that conditioned media from human abSAT and fmSAT reduced glucose uptake in human skeletal myocytes, suggesting that adipose tissue-induced alterations in skeletal muscle occur in the early stages of T2D.[23]

The inclusion of human fmSAT from study participants is a particularly unique aspect of this investigation, as fmSAT is underexplored. Previous investigations in humans have largely been confined to abSAT and VAT, leaving the metabolic influence of femoral adiposity insufficiently characterized. Many human studies have found that adipose tissue-conditioned media impair skeletal muscle glucose uptake.[17,17–19,23–25] Further regional breakdown of adipose tissue depots finds that VAT impairs glucose sensitivity more so than abSAT.[17,24,26] However, our study finds that abSAT and fmSAT impair glucose uptake with no effect from VAT. Only one study has examined the impact of human-derived regional adipose tissue conditioned media on glucose uptake *in vitro*, using the same 2-NBDG glucose uptake assay presented here.[24] In this study, the effects of media from human abSAT and VAT on glucose uptake in rat L6 cells were compared. Although findings showed that VAT, not abSAT-conditioned media, reduced skeletal muscle glucose uptake, the VAT-conditioned media did so only at dilutions of 1:64 and 1:128.[24] Interestingly, we found that abSAT and fmSAT impaired glucose uptake at a 1:1 dilution.

Our results contradict our original hypothesis that adipose tissue from abdominal depots, particularly VAT, would have the greatest negative impact on skeletal muscle glucose uptake.

The novel nature of our findings may be due to the first use of human female adipose tissue and myocytes. In all studies presented above, cross-species use of human adipose tissue with animal myocytes limits the applicability of findings. Future studies should use human adipose tissue and skeletal muscle samples to ensure the *in vivo* relevance and translational potential of the findings. Only one study was found that examined the mechanism of impaired glucose uptake in adipose tissue co-cultures. Sarr et al. report that in females, glucose signalling was impaired at the protein kinase B (Akt) phosphorylation sites, Akt Thr308 and Akt Ser473.[17]

Given that skeletal muscle structural alterations have been reported in individuals with T2D[4], it is plausible that changes in myocyte morphology could contribute to impaired glucose uptake. This study is the first to directly assess whether exposure to adipose tissue-conditioned media from the three main regional depots in individuals with obesity and T2D alters skeletal muscle MyHC thickness or fusion index. Our findings reveal that myocyte structure was unaffected by conditioned media from any adipose tissue depot. These results are consistent with a previous study reporting no change in young myocyte fusion index or thickness following

exposure to conditioned media from abSAT or VAT in individuals with obesity.[27] In contrast, Pellegrinelli et al.[15] reported reduced MyHC thickness following exposure to VAT-conditioned media. These results may differ from ours due to differences in skeletal muscle cell source, as myocytes were isolated from infants rather than adults. Collectively, these findings support the notion that altered skeletal muscle glucose uptake can occur independently of changes to myofiber structure.

A major strength of this study is the inclusion of all three principal adipose tissue depots in a human model, addressing a critical gap in the literature. Prior studies have largely relied on rodent models, examined effects on cells from different species, or focused exclusively on comparisons between abSAT and VAT. The use of adult human-derived adipose tissue and skeletal myocytes enhances the physiological relevance of our findings and improves their translational potential. Nevertheless, the present study has limitations. While we demonstrate clear depot-specific functional effects on glucose uptake, we did not quantify individual hormones, cytokines, or other bioactive factors secreted into the conditioned media. The primary aim of this study was to characterize the net functional effects of regional adipose tissue secretomes on skeletal muscle glucose uptake rather than to identify specific mediators. This integrated approach captures biologically relevant signalling complexity but limits mechanistic interpretation. Future studies should incorporate proteomic, lipidomic, and inflammatory profiling of conditioned media, along with targeted analysis of skeletal muscle glucose transport and insulin signalling pathways, to identify factors responsible for depot-specific differences.

In summary, our findings demonstrate that in females with obesity and T2D, abSAT and fmSAT, rather than VAT, impair glucose uptake in healthy skeletal muscle *in vitro*. These impairments occur independently of changes in myogenesis, suggesting that the effects of adipose tissue on skeletal muscle glucose uptake are distinct from muscle structural remodelling. Elucidating the molecular mediators underlying these depot-specific effects may lead to improved strategies for preventing and treating skeletal muscle insulin resistance in T2D.

Abbreviations

Abbreviation	Meaning
abSAT	Abdominal Subcutaneous Adipose Tissue
BMI	Body Mass Index
CIUSSS-NIM	Le Centre intégré universitaire de santé et de services sociaux du Nord-de-l'île-de-Montréal
CT	Computed Tomography
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
DXA	Dual-energy X-ray absorptiometry
fmSAT	Femoral Subcutaneous Adipose Tissue
HbA1c	Hemoglobin A1c or glycated hemoglobin
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
L2-3	Lumbar 2-3
MyHC	Myosin Heavy Chain
OB+T2D	Individuals living with obesity and type 2 diabetes
PBS	Phosphate Buffered Saline
T2D	Type 2 diabetes
VAT	Visceral Adipose Tissue
2-NBDG	2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose

Declarations

Ethics: Ethical approval was obtained from the University Human Research Ethics Committee of Concordia University, Comité d'éthique de la recherche du CIUSSS du Nord- de-l'Ile-de-Montreal, Hopital du Sacré-Coeur de Montreal, and Comité central d'éthique de la recherche du ministre de la Santé et des Services sociaux. All participants provided written informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for Publication: Not applicable.

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The authors have no competing interests to declare.

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Author Contribution: KZD, BT and SS were responsible for the conceptualization of the study. Adipose tissue and muscle biopsies were taken by JAM, MAT, RD, HA, RP, and PYG. KZD, JM and NK processed the biopsies. KZD and JD ran the experiments. Protocol guidance for cell cultures was provided by MEH. JL provided guidance on the protocol for the condition media.

KZD conducted the analysis and wrote the manuscript under the guidance of SS. All authors contributed to editing the final manuscript.

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Figures

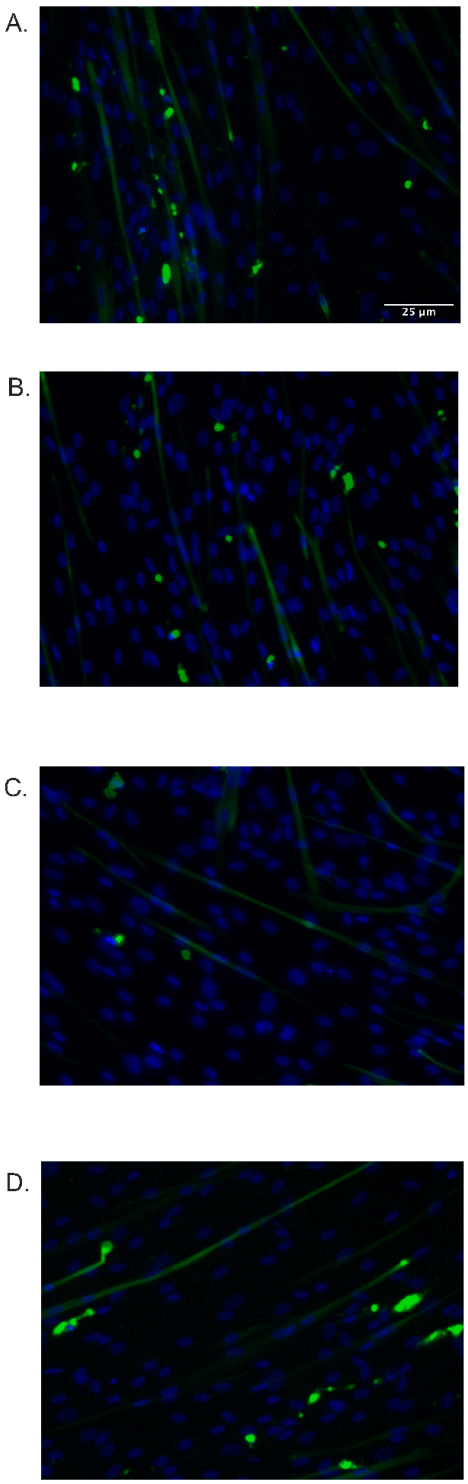


Figure 1

Examples of myosin heavy chain staining.

Images are of healthy skeletal muscle cells that have been exposed to regional adipose tissue conditioned media. Abdominal subcutaneous adipose tissue (A), femoral subcutaneous adipose tissue (B), visceral adipose tissue (C), control (D).

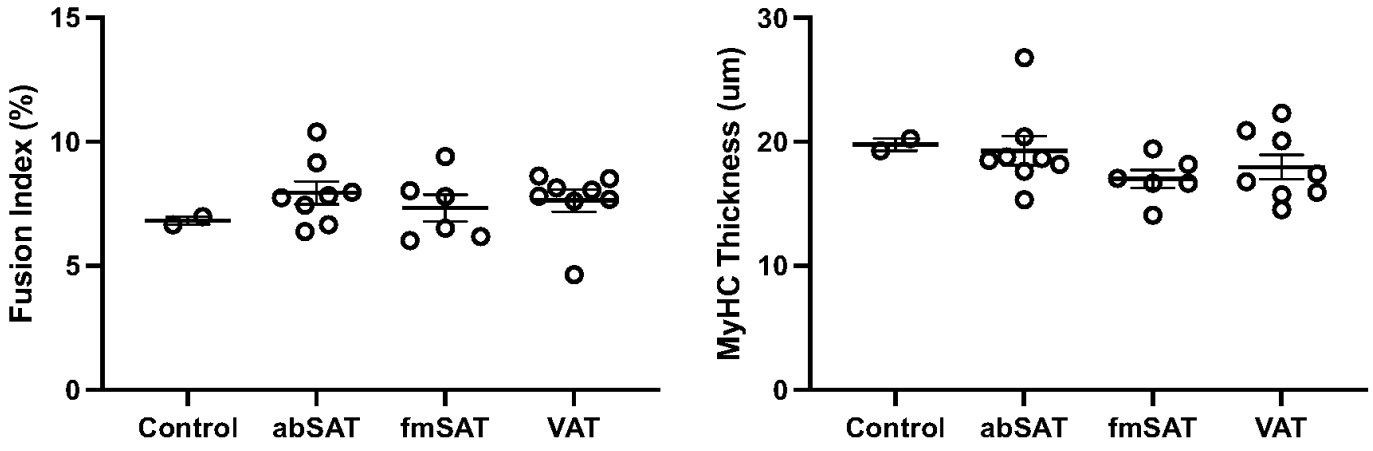


Figure 2

The effect of regionally conditioned media on glucose uptake.

2-NBDG glucose uptake for n=8 females. abSAT: abdominal subcutaneous adipose tissue. fmSAT: femoral subcutaneous adipose tissue. VAT: visceral adipose tissue. Each open circle represents a regional measure for each participant. Line represents mean ± SEM.

LSD post hoc detected between-group significance at *p<0.05, **p<0.01, **p<0.001.

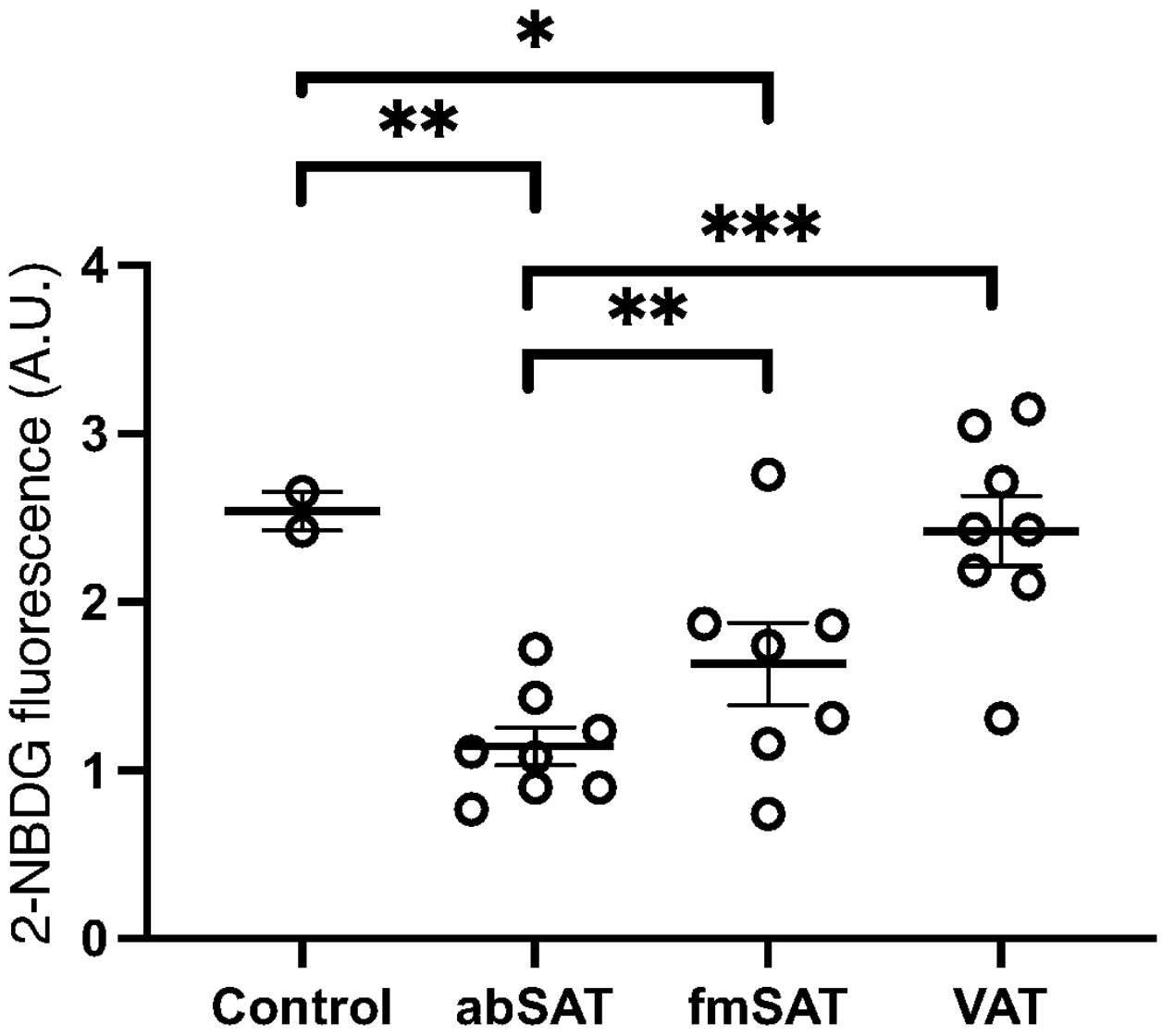


Figure 3

The effect of regional conditioned media on myogenesis.

A. Fusion index (%) for n=8 females. B. Myosin heavy chain thickness (um) for n=8 females. abSAT: abdominal subcutaneous adipose tissue. fmSAT: femoral subcutaneous adipose tissue. VAT: visceral adipose tissue. MyHC: myosin heavy chain. Each open circle represents a regional measure for each participant. Line represents mean ± SEM. LSD post hoc did not identify any between-group significance.