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BIOMEDICAL SCIENCES

Impact of Mesotherapy with Sodium Deoxycholate on Liver: Metabolic- and Sex-Specific Insights in Swiss mice

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Abstract: Sodium deoxycholate (DC) mesotherapy is approved for submental fat reduction but lacks evidence for body contouring safety in other body regions. Thus, we studied the systemic and hepatic metabolic effects of DC mesotherapy (50 µg/twice weekly for 4 weeks) in the inguinal white adipose tissue of female and male Swiss mice on a 20% fructose diet (drinking water) for 12 weeks. DC led to adipose tissue hemorrhage, foam cells, and fibrosis, although no body weight and adiposity loss, similar to humans. In males, glucose and hepatic metabolism, hepatic morphology, and protein expression (farnesoid X receptor and fibroblast growth factor-21) did not change by DC, even under fructose feeding. In females, although DC increased hepatic total cholesterol, most changes when detected were due to fructose (e.g., hepatic weight and lipid deposition). In conclusion, chronic DC mesotherapy proved safe for systemic and hepatic metabolism, and when adverse effects are present, they are sex-specific, impacting mostly females, especially under an unhealthy diet. Overall, care must be taken to extrapolate this data to humans since further studies are required to prove its safety in other body systems.

Key words: animal model, body contouring, deoxycholic acid, mesotherapy, metabolism, sodium deoxycholate.

INTRODUCTION

Mesotherapy, also called local intradermal therapy, was initially introduced in 1952 by a French doctor named Pistor (Mammucari et al. 2021). The technique promotes direct transepidermal transport of active substances into the dermis and deeper skin layers (Le Coz 2008, Mammucari et al. 2020). It has gained increasing popularity in aesthetics for localized fat removal since it is less invasive and expensive than liposuction (ISAPS 2020, Rittes 2003). Sodium deoxycholate (DC), or deoxycholic acid, is a secondary bile acid produced by the gut microbiota through cholic acid metabolism. It emulsifies dietary fats, increasing their superficial area to enzyme action and allowing their absorption (Gonçalves et al. 2020, Safari et al. 2020). Kybella is a proprietary form of DC, but compounding pharmacies can provide the same substance (Liu et al. 2019). In the United States, the use of DC has been approved since 2015, and until 2022, it was indicated solely for submental fat reduction in adults (Food and Drug Administration 2023). Likewise, Canada, Australia, Europe, and South Korea have approved DC use only for submental fat reduction (Humphrey et al. 2022). However, DC is widely used off-label in other body regions, such as the bra line and for body contouring (Zarbafian & Fabi 2020).

Consequently, studies need to be carried out to prove its safety, but only some ongoing clinical trials are investigating the metabolic impact of abdominal fat reduction by DC. Published data so far evaluated serum lipid and adipokine profile (Walker & Lee 2015a), DC safety and tolerability (Walker et al. 2015), and abdominal fat histopathology (Walker et al. 2020) without taking into account DC systemic effects in other organs.

Firstly, the potency of DC as a detergent on adipocytes made it the primary substance for fat reduction in mesotherapy. It permeates the adipocyte membrane, destabilizing and disintegrating the lipid bilayer, subsequently forming micelles composed of detergent and cell membrane lipids (Rotunda et al. 2004). It results in fat necrosis due to the leakage of the cytoplasmic content and the massive release of triglycerides (TG) into the interstitium (Ibáñez-Vicente et al. 2021), promoting macrophage recruitment to remove lipids and cellular debris (Metzger et al. 2020) and local inflammation (Walker et al. 2020). Nevertheless, it remains uncertain whether the lipids released reach the liver, a central organ in lipid homeostasis (Trefts et al. 2017). Under normal circumstances, the liver stores small amounts of TG in cytoplasmic lipid vacuoles. However, in scenarios of excessive circulating TG, such as overnutrition, obesity, and adipose tissue inflammation, the hepatic fatty acid metabolism is impaired, leading to hepatocyte TG accumulation (Alves-Bezerra & Cohen 2017). Overall, we hypothesize that DC promotes an overflow of TG from adipose cells to the liver, leading to hepatic TG storage and tissue dysfunction.

A second issue is that DC has tissue receptors in organs enrolled in glucose and lipid metabolism (Kumar et al. 2016, Zhang et al. 2022, Zhou & Anakk 2022). In humans, it has been proved that DC reaches the bloodstream after subcutaneous injection. Walker & Lee (2015a) showed that DC 100 mg sc increases DC serum levels within the endogenous normal range, and after 12 hours, it returned to basal levels; thus, DC might have a systemic action through its receptors. Moreover, DC binds to several tissues that express its receptors and may be implicated in a series of metabolic diseases (Jovanovich et al. 2022, Zhang et al. 2022). With this in mind, we hypothesize that the chronic use of DC in mesotherapy modulates the hepatic metabolism and gene expression through the farnesoid X receptor (FXR) (Copple & Li 2016). Finally, the fibroblast growth factor 21 (FGF21), which connects the liver with the adipose tissue and prevents hepatic lipid deposition, might also be impacted by DC mesotherapy (Szczepańska & Gietka-Czernel 2022).

Overall, we investigated the systemic and hepatic effects of chronic use of DC, administered multiple times subcutaneously over subsequent weeks in mesotherapy for fat reduction and its potential impact on hepatic fat metabolism. Male and female Swiss mice were studied under fructose consumption to investigate the role of sex and diet on the outcomes under investigation.

ABBREVIATIONS

ΔBW	BW variation
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BW	Body Weight
CEUA	Ethics Committee for Animal Use
DC	Sodium deoxycholate
eWAT	Epididymal white adipose tissue
F	Fructose
FGF21	Fibroblast growth factor
FXR	Farnesoid X receptor
GGT	Gamma-glutamyl transferase
HDL	High-density lipoprotein

HE	Hematoxylin and eosin
LDL	Low-density lipoprotein
pWAT	Parametrial white adipose tissue
riWAT	Right inguinal white adipose tissue
TC	Total cholesterol
TG	Triglycerides
UFF	Universidade Federal Fluminense

MATERIALS AND METHODS

Ethics

The Ethics Committee for Animal Use (CEUA) of the Universidade Federal Fluminense (UFF) approved the protocol (CEUA/UFF 4232071220/2021). The investigation respected the Guide for the Care and Use of Laboratory Animals (Committee 2011) and the Brazilian Guide for the Production, Maintenance, or Use of Animals in Teaching or Research Scientific Research Activities (Concea 2023).

Animals

Female and male Swiss mice were provided by the Central Animal Care Facility (UFF, Niteroi, RJ, Brazil). Swiss mice were chosen because, in our personal experience, they have a larger subcutaneous fat depot than other mouse strains in normal-weight mice. They were maintained in the Animal Care Facility of the Instituto de Saúde de Nova Friburgo (UFF, Nova Friburgo, RJ, Brazil) and housed under controlled temperature (21±1°C), humidity (60±10%), and light/dark cycle (12 h/12 h lights on at 7 a.m.). They had free access to water and food (Nuvilab CR-1, Quimtia, PR, Brazil), which were recorded daily, and body weight (BW) was recorded weekly using an electronic precision weighing scale (LS1, Marte Científica, MG, Brazil). Body weight variation (ΔBW) during DC intervention was calculated by subtracting the BW of the 12th week from the BW of the eighth week of the experiment.

Study design

Our study was conducted over twelve weeks. Twelve-week-old female (n=29) and male (n=30) Swiss mice were given 20% fructose (F) diluted in filtered water (fructose(D) P.A, Labsynth, Diadema, SP, Brazil) or solely filtered water for twelve weeks in their water bottle. From the eighth week onwards, the mice also received subcutaneous injections of saline (100 µL) or 50 µg DC (Sigma, D6750) diluted in 100 µL saline twice weekly on the right inguinal white adipose tissue (riWAT) depot. Thus, DC was administered for four weeks at 100 µg/week, and the solution pH was 7.0. DC dose was set in a pilot study in which a single sc injection of 13 µg and 130 µg DC reproduced the WAT inflammation phenotype described in humans in a dose-dependent manner (unpublished data). Since the current protocol of DC mesotherapy would last four weeks, we chose to administer 100 µg fractionated twice weekly to ensure the animal's welfare.

In awake mice fasted for six hours, a small blood drop was obtained from the tail tip and used for glucose assessment (ACCU-Chek Performa, Roche, Jaguaré, SP, Brazil). Then, mice were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine ip), blood was collected by cardiac puncture, centrifuged, and the serum was stored at -20 °C. The liver, riWAT, and the right and left epididymal (eWAT, male) and parametrial (pWAT, females) genital WAT were harvested (Giordano et al. 2022), weighed (Shimadzu AUX220, Kyoto, Japan), and fixed in 4% buffered formalin or stored at -80 °C. The left tibia was removed and measured to correct liver weight (g/cm) (Yin et al. 1982).

Blood and tissue biochemistry

Serum TG (K117, Bioclin, MG, Brazil), total cholesterol (TC) (Interkit, Belo Horizonte, MG, Brazil), glycerol (K015/K117, Bioclin, MG, Brazil),

albumin (Interkit, Belo Horizonte, MG, Brazil), and direct bilirubin (Interkit, Belo Horizonte, MG, Brazil) were analyzed by colorimetric assays, and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) (Interkit, Belo Horizonte, MG, Brazil) by kinetic assays. The liver (~30 mg) was homogenized in 1 mL isopropyl alcohol (A1078.01. BJ, Labsynth, Diadema, SP, Brazil), centrifuged at 1.132 x g (NT805, Nova Técnica, Piracicaba, SP, Brazil), and the supernatant reserved for hepatic TG, TC and glycerol assays. Assays were adapted for use in a 96-well microplate, and the wavelength was read in a microplate spectrophotometer (Epoch™, BioTek, Vermont, USA). The fasting glucose and TG product (TyG index) was calculated as previously described to investigate insulin resistance (Simental-Mendía et al. 2008).

Qualitative and quantitative histopathology

Right inguinal WAT samples followed the routine histological processing for formalin-fixed paraffin-embedded tissues, were sectioned at 5 µm thick, and stained with hematoxylin and eosin (HE) and Masson's trichrome. Qualitatively, we investigated riWAT hemorrhage, inflammation, macrophage infiltration, and fibrosis, whereas quantitatively, we assessed adipocyte diameter (Gonçalves et al. 2017).

Liver samples followed the same histological procedure. Liver hepatocyte nuclei density and hypertrophy were investigated in HE-stained sections by the number density of hepatocyte nuclei ($Q_{A[nuclei]}$) and the volume-weighted mean nuclear volume ($\bar{V}_{V[nuclei]}$), respectively (Marcos et al. 2012). Hepatic steatosis was analyzed by pointing counting (volume density, $V_{v[lipid\ droplets]}$) in frozen liver samples embedded in optimal cutting temperature (OCT) compound, sectioned at 10 µm thick, and stained with Oil red O (Catta-Preta et al. 2011, Mehlem et al. 2013).

Digital images were acquired on an Opticam O400S microscope and a digital camera OPTHD 5.3MP (Opticam Microscopy Technology, SP, Brazil). Random non-consecutive sections were used, and 8-10 digital images (1,296 x 972 pixels) were acquired per animal. Adipocyte morphometry was performed in the OPTHD Microscope Image Software (v. 4.11.21849.20221208, Opticam Microscopy Technology, SP, Brazil), and stereology on STEPanizer (Tschanz et al. 2011), except for $\bar{V}_{V[nuclei]}$ whose test system was created in Fiji ImageJ (https://imagej.net/software/fiji/).

Western blot

Total proteins were extracted from ~150-200 mg liver (RIPA lysis buffer). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10 % polyacrylamide gel) and transferred to a polyvinylidene fluoride (PVDF) membrane (RNP303F, GE Healthcare, Little Chalfont, Buckinghamshire, UK) in a semi-dry blotter (TE70X, Amershan Hoefer, Holliston, MA, USA). Membranes were incubated in 5% non-fat dry milk for 1 h and then overnight with primary antibody anti-FGF21 (1:1,000, rabbit, SAB2108136, Sigma), anti-FXR (1:1,000, mouse, 72105S, Cell Signaling), and anti-β-actin (internal control, 1:5,000, mouse, sc-2005, Santa Cruz). The following day, membranes were washed with tris-buffered saline tween buffer (T-TBS), incubated in anti-rabbit (1:2,500, PI-1000-1, Vector Labs) or anti-mouse (1:2,500, PI-2000-1, Vector Labs) secondary antibodies, and the reaction was visualized by enhanced chemiluminescence (ECL, ThermoFisher, Pierce™ ECL Western Blotting Substrate, Carlsbad, CA, USA) using a chemiluminescence analyzer (c-DiGit Blot Scanner, LI-COR). The Image Studio Lite Software v. 5.0 (LI-COR) quantified the relative optical density.

Statistical analysis

Data are presented as mean ± standard deviation (S.D.). The Shapiro-Wilk test analyzed data distribution and variance homoscedasticity. The two-way ANOVA analyzed the effect of the independent factors DC injection and fructose consumption and its interaction on dependent variables, and the Holm-Sidak post hoc test was used for multiple comparisons ($p<0.05$, GraphPad Prism v. 8 for Windows, Boston, MA, USA).

RESULTS

DC-induced inflammation and fibrosis in riWAT without fat or body weight loss

In females, fructose increased BW progressively, but not in males (Fig. 1a-b). On the other hand, chronic use of DC had no impact on the BW of female and male mice in the DC and F/DC groups, compared to the C and F groups, respectively (Fig. 1a-b). Similarly, BW variation (ΔBW) during the treatment period (8-12 weeks) did not show any change in either females or males (Fig. 1c-d). Likewise, the riWAT weight, which was the

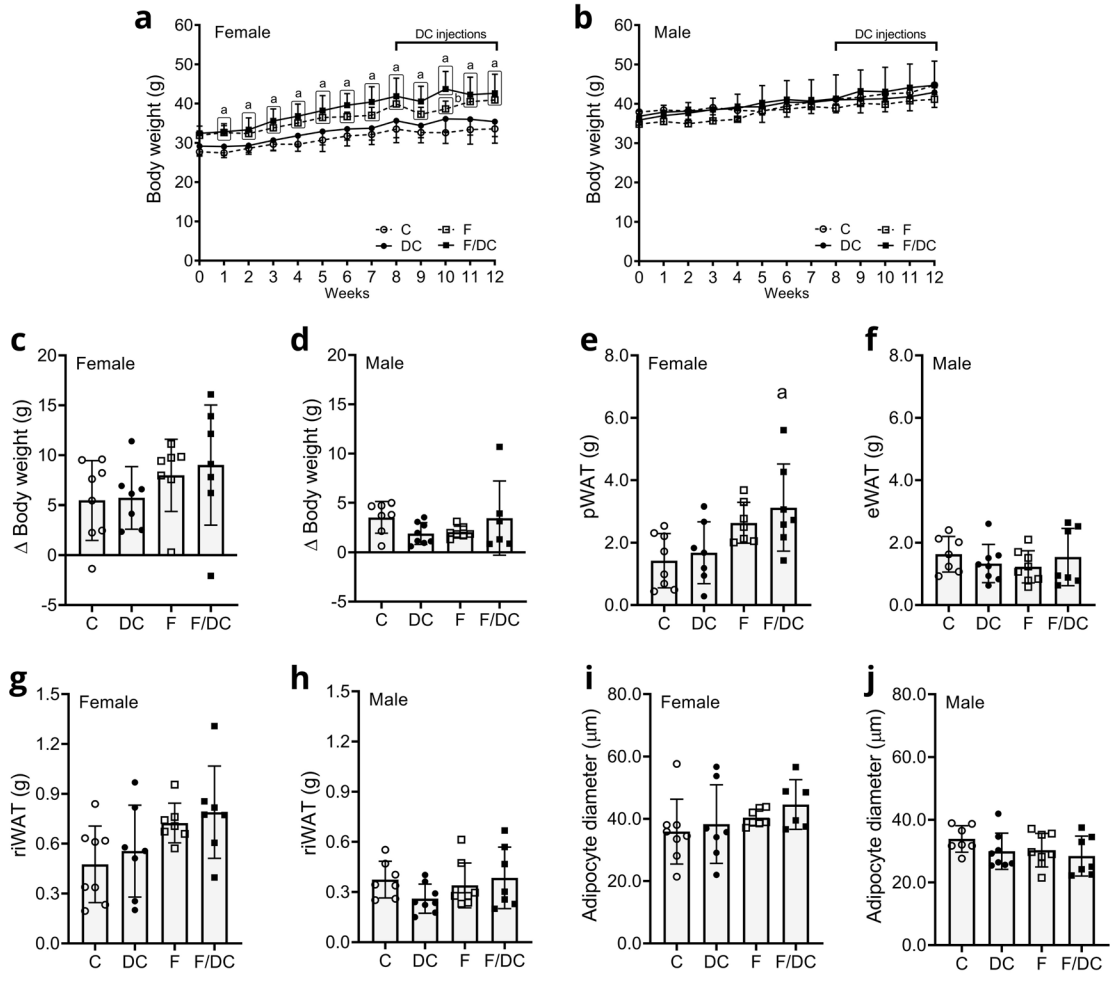

Figure 1. Effect of subcutaneous injections of sodium deoxycholate (DC) on body weight and adiposity in female and male Swiss mice. 1a-b, Body weight. 1c-d, Body weight gain from weeks 8 to 12. 1e, Parametrial white adipose tissue (pWAT) weight. 1f, Epididymal white adipose tissue (eWAT) weight. 1g-h, Right inguinal white adipose tissue (riWAT) weight. 1i-j, Adipocyte diameter. Data are shown as mean ± S. D., $p<0.05$, [a] vs. C, [b] vs. F. Groups: C, control; DC, sodium deoxycholate; F, fructose; F/DC, fructose + sodium deoxycholate.

site of DC administration, was not affected by DC (Fig. 1g-h). We assessed gonadal fat weight (parametrial in females and epididymal in males) since it correlates with body fat percentage in mice (Oldknow et al. 2015). Interestingly, while eWAT showed no change in males (Fig. 1f), pWAT increased in the F/DC group compared to C in females (+119%, p=0.0016, Fig. 1e).

The histopathological analysis showed no alterations in adipocyte diameter in females and males (Fig. 1i-j and Supplementary Material – Fig. S1a-d and S2a-d). Chronic DC injections promoted riWAT inflammation, hemorrhage, and fibrosis in both sexes regardless of fructose feeding, as shown in Fig. S1e-j and S2e-h. Hemorrhage was observed among the adipose cells, and crown-like structures consisting of foam cells (macrophages) surrounding dead adipocytes were also present. Fibrosis was characterized by collagen fibers surrounding the riWAT depot or collagen bundles among adipocytes. Of note, adipocyte diameter was analyzed in the vicinity of sites of inflammation and fibrosis in DC and F/DC groups, not representing, for instance, dying adipocytes surrounded by inflammatory cells such as macrophages.

Sex-specific effects of chronic DC on serum and liver biomarkers

Several serum and tissue biomarkers were examined to explore the role of DC on glucose, lipid, and hepatic metabolism (Table I). Interestingly, DC could not change any glycemic parameter (glucose and TyG index) in female and male mice. Likewise, TG, TC, and glycerol (an indirect marker of lipolysis) remained similar to the C group in female and male mice receiving DC (DC and F/DC groups). DC did not alter the

Table I. Serum and hepatic biochemistry.

Parameters	Female				Male			
	C	DC	F	F/DC	C	DC	F	F/DC
Serum								
Glucose, mg/dL	135.0 ± 3.6	133.1 ± 5.2	148.0 ± 4.5	133.4 ± 4.2	151.8 ± 14.4	146.7 ± 24.6	128.1 ± 22.6	154.5 ± 53.4
TyG index	8.35 ± 0.54	8.42 ± 0.45	8.86 ± 0.26	8.58 ± 0.37	4.89 ± 0.22	4.76 ± 0.22	4.61 ± 0.33	4.76 ± 0.24
TG, mg/dL	70.7 ± 32.6	75.4 ± 37.4	97.6 ± 22.4	84.8 ± 31.4	119.9 ± 42.5	107.5 ± 37.3	93.0 ± 40.0	105.8 ± 36.0
Glycerol, mg/dL	51.4 ± 14.0	42.4 ± 10.8	45.0 ± 5.6	53.2 ± 30.4	493.4 ± 182.5	320.5 ± 286.1	371.0 ± 271.4	429.1 ± 336.4
TC, mg/dL	165.1±10.3	152.5±22.0	185.4±30.7	170.7±23.6	239.6±30.0	233.7±36.9	213.1±48.7	214.6±34.3
ALT, IU	4.63 ± 1.67	4.39 ± 1.14	4.31 ± 2.55	3.05 ± 2.16	7.81 ± 0.96	5.46 ± 0.95a	3.66 ± 0.67[a,b]	5.30 ± 1.80[a]
AST, IU	7.86 ± 1.67	7.49 ± 1.63	6.57 ± 1.65	7.90 ± 1.98	8.98 ± 3.66	7.97 ± 1.37	8.11 ± 1.65	7.81 ± 2.76
GGT, IU	4.01 ± 0.91	3.83 ± 0.82	4.46 ± 2.08	3.44 ± 2.03	3.93 ± 0.63	3.25 ± 1.68	3.50 ± 0.98	3.01 ± 1.82
Albumin, mg/dL	2.31 ± 0.26	2.13 ± 0.25	2.13 ± 0.27	1.94 ± 0.26	1.74 ± 0.11	1.62 ± 0.07[a]	1.49 ± 0.07[a,b]	1.42 ± 0.09[a]
Bilirubin, mg/dL	0.48 ± 0.15	0.54 ± 0.16	0.51 ± 0.15	0.52 ± 0.12	0.93 ± 0.59	0.86 ± 0.10	0.52 ± 0.50	0.57 ± 0.47
Liver								
TG, mg/g	31.3±8.2	33.8±8.1	36.3±6.1	40.1±6.5	2.7±1.9	4.9±3.8	6.4±3.7	6.5±5.7
Glycerol, mg/g	24.3±4.7	24.3±5.9	28.0±4.2	24.8±7.0	nd	nd	nd	nd
TC, mg/g	13.2±5.7	22.8±7.8	46.4±21.0 [a]	92.1±24.7 [a,b,c]	31.6±7.4	35.3±13.3	37.0±11.7	36.8±11.8

Data are expressed as mean ± S. D. When indicated, p<0.05, [a] C, [b] DC, [c] F, two-way ANOVA, and Holm-Sidak post hoc test. Abbreviations: C, control. DC, sodium deoxycholate. F, fructose. TyG, triglyceride-glucose index. TC, total cholesterol. TG, triglyceride. ALT, alanine aminotransferase. AST, aspartate aminotransferase. GGT, gamma-glutamyl transferase (GGT). nd, not detected.

serum markers of liver injury ALT, AST, and GGT in females. Still, in males, both DC and F reduced ALT compared to the C group (DC -30.1%, F -53.2%, and F/DC -32.1%, p<0.0001), and this parameter was influenced by F and F/DC interaction (p<0.0001, two-way ANOVA). Finally, bilirubin did not change, and albumin levels decreased in male DC, F, and F/DC groups compared to the C group and were influenced by DC and its interaction with F (p<0.0001, two-way ANOVA).

Hepatic TG and TC analysis revealed no impact of chronic DC use on tissue metabolism in male mice, like TG and glycerol response in females. Unfortunately, glycerol was below the range of detection in males. Finally, hepatic TC in female mice showed a considerable increase in the F/DC group compared to all other groups (C +598% p<0.0001, DC +304% p<0.0001, F +98% p<0.01), and also in the F group compared to the C (+252%, p<0.05). This parameter was influenced by DC (p=0.0012) and F (p<0.0001) and their interaction (p<0.02, two-way ANOVA).

DC did not change liver mass, hepatocyte size, or protein expression

Fructose, but not DC, increased liver mass in female F and F/DC groups compared to the C group (Fig. 2a). No change was seen in male liver weight (Fig. 2b). Then, we assessed the number density of hepatocyte nuclei and the volume-weighted mean nuclear volume since they are indirect and direct measurements, respectively, of hepatocyte hypertrophy. In female mice, the number density of nuclei was not changed by DC (Fig. 2c), while a greater nuclei volume was seen in the F group (+53.0%, p<0.0001, Fig. 2e). In male mice, the number density of hepatocytes and the nuclei volume showed no change, as shown in Fig. 2d and 2f, respectively.

Furthermore, DC did not promote hepatic fat deposition, but fructose had some impact on females, not males. In females, F and F/DC

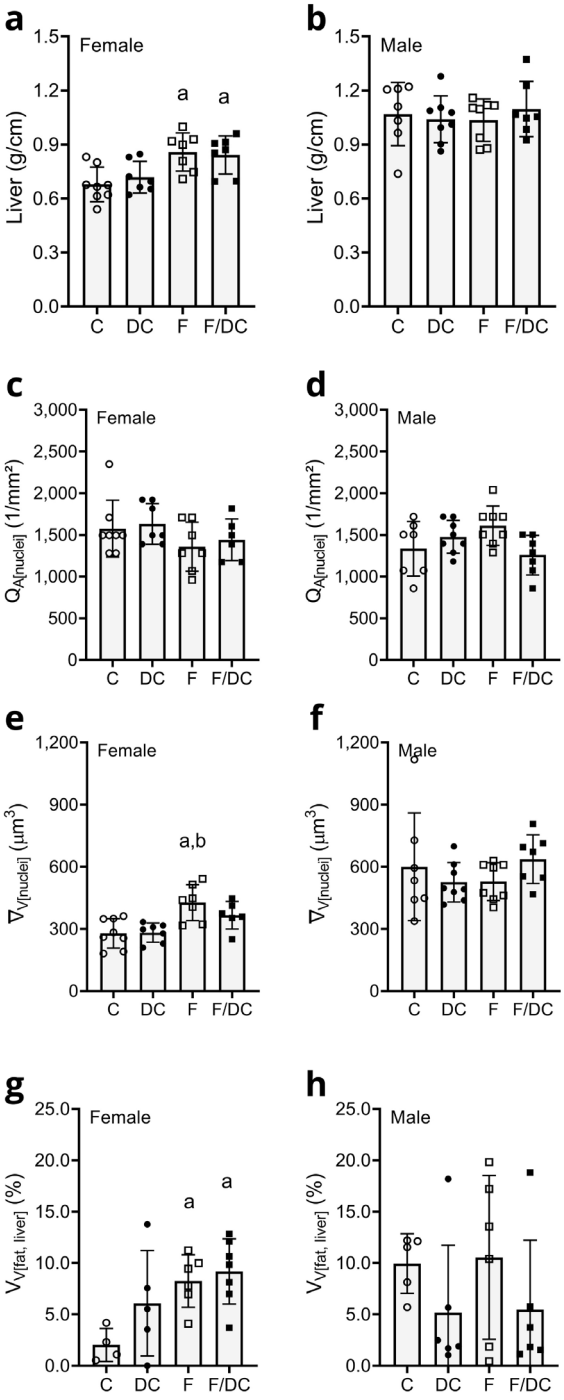

Figure 2. Liver mass and quantitative histopathology in female and male Swiss mice. 2a-b, Liver mass. 2c-d, Number density of hepatocytes ($Q_{A[nuclei]}$). 2e-f, Volume-weighted mean nuclear volume ($\bar{V}_{V[nuclei]}$) of hepatocytes. 2g-h, Volume density ($V_{v[lipd\ droplets]}$) of hepatic fat. Data are shown as mean ± S. D., p<0.05, [a] vs. C, [b] vs. DC. Groups: C, control; DC, sodium deoxycholate; F, fructose; F/DC, fructose + sodium deoxycholate.

groups showed a higher degree of hepatic fat deposition compared to the C group (F +305.3%, F/DC +351.4%, p<0.005, Fig. 2g), evidenced by liver photomicrographs stained with Oil red (Fig. 3). On the opposite way, male's liver showed no change in fat deposition (Figs. 2h). Finally, FXR protein expression, a major regulator of bile acids, did not change by DC in females and males (Fig. 4a-b), similar to FGF21, which is involved in free fatty acid β-oxidation (Fig. 4c-d).

DISCUSSION

In Humans, DC promotes the local lysis of adipocyte cell membrane, triggering an inflammatory cascade (Muskat et al. 2022, Rose & Morgan 2005) that takes approximately 28 days to resolve completely (Walker et al. 2020). Non-invasive procedures, such as cryolipolysis, trigger adipocyte apoptosis, preserving the adjacent structures, but unlike DC, the inflammatory process takes three months to resolve completely (Klein et al. 2017). Some side effects are observed at the application

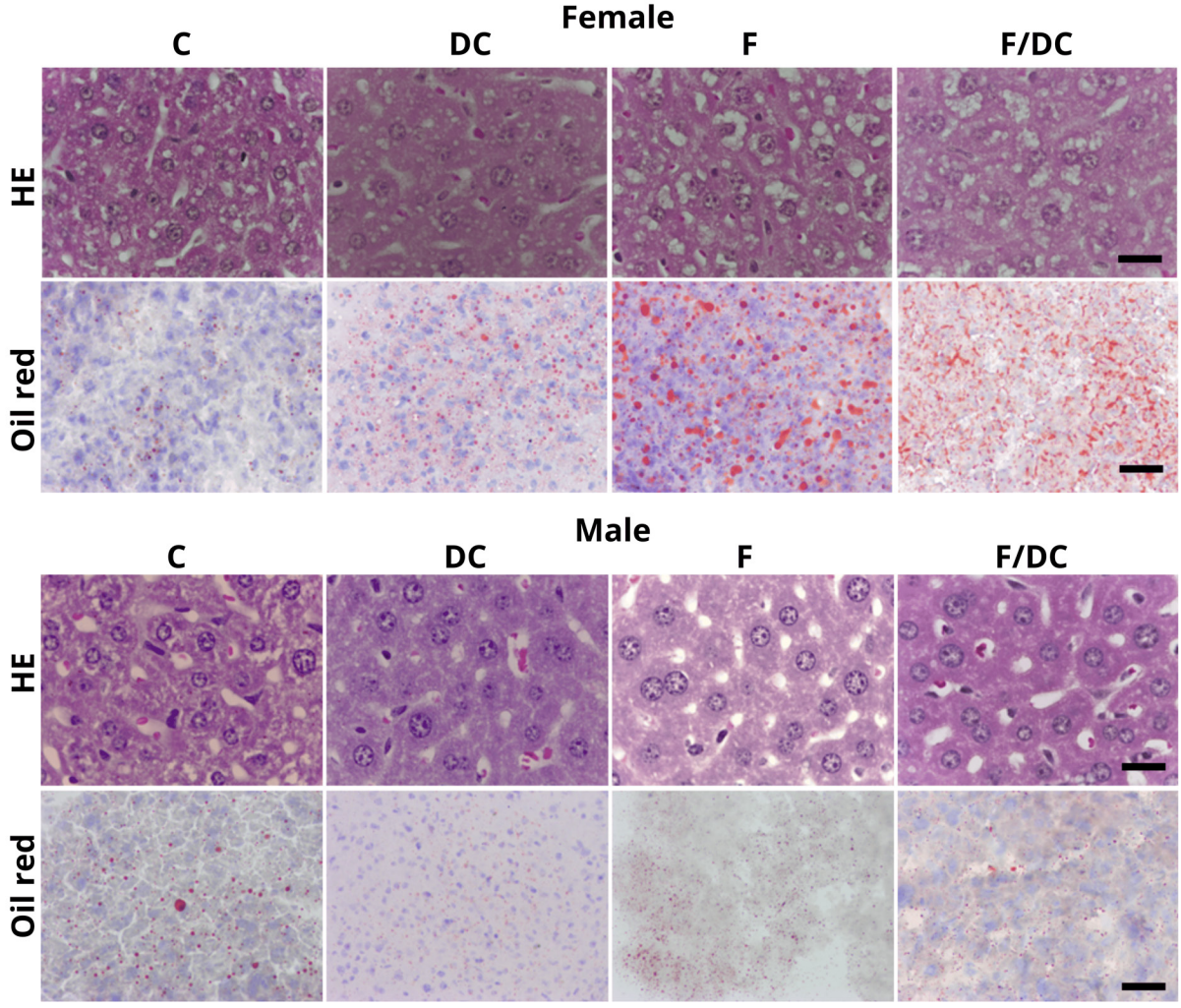

Figure 3. Representative liver photomicrographs stained with hematoxylin and eosin (HE) and Oil red O. Groups: C, control; DC, sodium deoxycholate; F, fructose; F/DC, fructose + sodium deoxycholate. HE, hematoxylin and eosin. Bar = 60 µm.

site, such as pain, swelling, numbness, bruising, induration, nodules, and itching (Farina et al. 2020, Jegasothy 2018, Sharpe et al. 2022). Also, DC-induced adipocyte destruction reduces body measurements but does not promote BW loss (Zarbafian & Fabi 2020). We successfully reproduced DC-induced riWAT inflammation without BW loss in female and male Swiss mice in the present study.

In addition, DC mesotherapy in humans is unable to permanently alter serum free fatty acids, TC, low-density lipoprotein (LDL), high-density lipoprotein (HDL), TG, and pro-inflammatory cytokines (Dayan et al. 2016, Walker & Lee 2015a). Cryolipolysis is also unable to alter lipid and liver parameters (Badran et al. 2023, Klein et al. 2017), while liposuction leads to a decrease in leptin, TG, and glucose

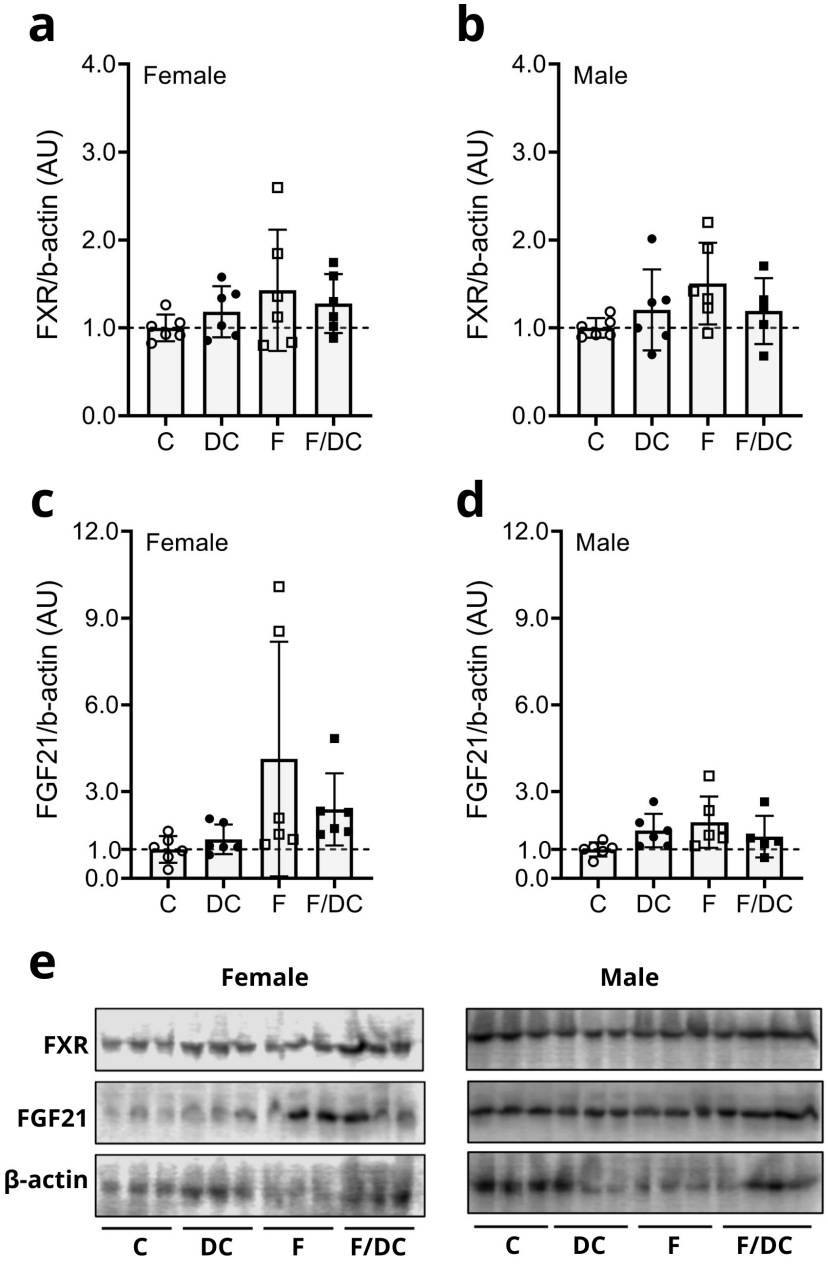

Figure 4. Liver protein expression in female and male Swiss mice. 4a-b, Farnesoid X receptor (FXR). 4c-d, Fibroblast growth factor 21 (FGF21). Groups: C, control; DC, sodium deoxycholate; F, fructose; F/DC, fructose + sodium deoxycholate. Abbreviations: AU, arbitrary units for relative protein expression.

(Gómez-Sámano et al. 2023). Our study could ratify previous evidence since we could not show long-term metabolic impairments in glucose, lipolysis, and hepatic enzymes. The only exceptions were ALT and albumin in males, whose levels were decreased in DC and F/DC groups, and hepatic TC in females, suggesting a sex-specific effect regardless of fructose consumption. In humans, low ALT levels are associated with a higher risk of mortality (Oren 2014), and hypoalbuminemia is an essential marker of the severity of body inflammation and physiological stress (Soeters et al. 2019).

Finally, we applied morphometric and stereological tools to investigate if DC would change liver morphology and quantified the relative protein expression of FXR and FGF21 by western blot, and we demonstrated a lack of adverse role of DC on these parameters. To date, it is the first study investigating the role of chronic DC use in mesotherapy in a mouse model focusing on hepatic outcomes and comparing sexes, which limits our discussion due to the need for additional evidence from other research groups.

DC-induced adipocyte membrane rupture releases a massive amount of TG into the interstitium, and macrophages are recruited to remove these lipids (Ibáñez-Vicente et al. 2021), as evidenced by the presence of foam cells (macrophages) in crown-like structures surrounding adipocyte cells (Gugliucci 2022). Hypothetically, TG phagocytosis by macrophages might prevent TG from freely entering the bloodstream (Poletto 2017). However, the extent to which macrophages can deal with this TG overload is still unknown, especially in the long term after successive DC injections. The question remains regarding where these lipids go and whether macrophages can phagocyte all of them. Thus, if DC is meant for body contouring, it is essential to establish safe maximum doses that can reduce subcutaneous fat without leading to massive WAT TG release.

In recent years, it has been discovered that bile acids such as DC can bind to multiple tissue receptors and act as metabolic regulators of pathways related to glucose, lipids, and amino acids metabolism, homeostasis maintenance, and gut microbiota, which explain their implication in several metabolic disorders (Jovanovich et al. 2022, Zhang et al. 2022). In the liver, DC binds to FXR, which regulates glycolipid metabolism and endothelial function, which are enrolled in cardiovascular disease risk (Zhang et al. 2022). In turn, FXR activation reduces circulating DC (Jovanovich et al. 2018) because it acts as a bile acid sensor in the enterohepatic circulation, and it maintains bile acid homeostasis by modulating the expression of genes enrolled in bile acid synthesis, transport, and excretion (Copple & Li 2016). FXR is a secondary receptor, with TGR5 being the principal DC receptor (Wang et al. 2021), which could explain why no changes were seen in FXR receptor expression.

Glycerol is an indirect biomarker of lipolysis that can be transported from the adipose tissue into the liver to be stored as TG. In addition, the liver can store TG under stress conditions such as abnormal lipid metabolism, high-fat high-carbohydrate intake, and obesity (Nassir et al. 2015). We did not observe changes in glycerol and TG levels induced by DC, which corroborates with FGF21 protein expression. Among its functions, FGF21 stimulates the β-oxidation of free fatty acids and suppresses the formation of TG and adipose tissue lipolysis, reducing TG's intrahepatic and serum content (Szczepańska & Gietka-Czernel 2022). Although we have shown that fructose feeding led to mild fat deposition in the liver of female mice, corroborating an unbalanced diet intake, it was not sufficient to modulate hepatic FGF21 protein expression.

Non-surgical fat reduction ranks fourth among men's most common non-surgical procedures and fifth among women (ISAPS 2020). Firstly, it surprisingly shows that the search for aesthetic procedures for fat reduction is not limited to women, and secondly, it highlights the relevance of performing studies investigating both sexes. As in humans, studies in animal models have already shown sex-specific effects, mediated by sex hormones, on adipocyte development, adipogenesis, lipogenesis, lipolysis, and insulin sensitivity (Chang et al. 2018). Thus, sex hormones may impact the body's response to DC mesotherapy, as well as to other substances that may be under investigation for body fat reduction.

We chose the Swiss mice for DC mesotherapy since the healthy mice have a large subcutaneous fat compartment at the inguinal region (Frontini & Cinti 2010). It is easily accessible for the administration of substances, and we have succeeded in DC administration at the riWAT. We also offered fructose to investigate the impact of DC mesotherapy on an organism fed with an unhealthy diet since fructose administration has been widely used to induce obesity and the metabolic syndrome phenotype in animal models (Zubiría et al. 2017). In Swiss mice, most studies offer fructose in drinking water, ranging from 15% to 30% (De Souza et al. 2021, Gambaro et al. 2018, 2020). According to Johnson et al. (2007), 10% fructose would induce hypertension and microvascular renal changes. Based on this evidence, we offered an intermediate dose of 20% fructose in drinking water for 12 weeks. Unfortunately, we did not find expressive BW gain and metabolic changes in fructose-feed mice of both sexes, which may be a limitation of this study.

In conclusion, DC did not impact BW or adipose tissue mass but promoted adipocyte lysis and inflammation, evidenced by riWAT hemorrhage, foam cells, and tissue fibrosis, even without affecting adipocyte size. Surprisingly, the chronic usage of DC had no impact on glucose and hepatic metabolism, hepatic morphology, and protein expression in males, even in mice under fructose feeding. However, it is important to note that hepatic TC and fat deposition increased considerably in females submitted to DC plus fructose, raising an alert to the use of DC in subjects under an unhealthy diet and the sex-specific role of these interventions. Thus, in the present mice model, chronic DC mesotherapy proved safe for male liver metabolism and should be used cautiously in females with a diet rich in fructose. Overall, care must be taken to extrapolate this data to humans since further studies are required to prove its safety in other body systems, such as the heart and kidneys.

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REFERENCES

ALVES-BEZERRA M & COHEN DE. 2017. Triglyceride metabolism in the liver. Compr Physiol 8(1): 1-8.

BADRAN S, DOI SA, ISKEIRJEH S, ALJASSEM G, JAFARIAN N, CLARK J, HABIB AM & GLASS GE. 2023. Metabolic changes after nonsurgical fat removal: A dose response meta-analysis. J Plast Reconstr Aesthet Surg 77: 68-77.

CATTA-PRETA M, MENDONCA LS, FRAULOB-AQUINO J, AGUILA MB & MANDARIM-DE-LACERDA CA. 2011. A critical analysis of three quantitative methods of assessment of hepatic steatosis in liver biopsies. Virchows Arch 459(5): 477-485.

CHANG E, VARGHESE M & SINGER K. 2018. Gender and sex differences in adipose tissue. Curr Diab Rep 18(9): 69.

COMMITTEE, NATIONAL RESEARCH COUNCIL. 2011. Guide for the care and use of laboratory animals, 8th ed., Washington: The National Academies Press, 220 p.

CONCEA. 2023. Guia brasileiro de produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica, 1st ed., Brasilia: Ministério da Ciência, Tecnologia e Inovação, 1107 p.

COPPLE BL & LI T. 2016. Pharmacology of bile acid receptors: Evolution of bile acids from simple detergents to complex signaling molecules. Pharmacol Res 104: 9-21.

DAYAN SH, HUMPHREY S, JONES DH, LIZZUL PF, GROSS TM, STAUFFER K & BEDDINGFIELD FC. 2016. Overview of ATX-101 (deoxycholic acid injection): A nonsurgical approach for reduction of submental fat. Dermatol Surg 42(Suppl 1): S263-S270.

DE SOUZA L, BARROS WM, DE SOUZA RM, DELANOGARE E, MACHADO AE, BRAGA SP, ROSA GK, NARDI GM, RAFACHO A & SPERETTA GFF ET AL. 2021. Impact of different fructose concentrations on metabolic and behavioral parameters of male and female mice. Physiol Behav 228: 113187.

FARINA GA, CHERUBINI K, DE FIGUEIREDO MAS & SALUM FG. 2020. Deoxycholic acid in the submental fat reduction: A review of properties, adverse effects, and complications. J Cosmet Dermatol 19(10): 2497-2504.

FOOD AND DRUG ADMINISTRATION. 2023. FDA-approved drugs: Kybella. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=206333. Accessed 2024/12/02.

FRONTINI A & CINTI S. 2010. Distribution and development of brown adipocytes in the murine and human adipose organ. Cell Metab 11(4): 253-256.

GAMBARO SE, ZUBIRÍA MG, GIORDANO AP, PORTALES AE, ALZAMENDI A, RUMBO M & GIOVAMBATTISTA A. 2020. Spexin improves adipose tissue inflammation and macrophage recruitment in obese mice. Biochim Biophys Acta Mol Cell Biol Lipids 1865(7): 158700.

GAMBARO SE, ZUBIRÍA MG, PORTALES AE, REY MA, RUMBO M & GIOVAMBATTISTA A. 2018. M1 macrophage subtypes activation and adipocyte dysfunction worsen during prolonged consumption of a fructose-rich diet. J Nutr Biochem 61: 173-182.

GIORDANO A, CINTI F, CANESE R, CARPINELLI G, COLLELUORI G, DI VINCENZO A, PALOMBELLI G, SEVERI I, MORETTI M & REDAELLI C ET AL. 2022. The adipose organ is a unitary structure in mice and humans. Biomedicines 10(9): 2275.

GONÇALVES LF, TORRES VM & FERNANDES-SANTOS C. 2020. Is there metabolic risk associated with the use of deoxycholic acid for enzyme liposuction? Rev Bras Estet Cient (1): 9-23.

GONÇALVES LF, MACHADO TQ, CASTRO-PINHEIRO C, DE SOUZA NG, OLIVEIRA KJ & FERNANDES-SANTOS C. 2017. Ageing is associated with brown adipose tissue remodelling and loss of white fat browning in female C57BL/6 mice. Int J Exp Pathol 98(2): 100-108.

GÓMEZ-SÁMANO M ET AL. 2023. Effect of liposuction on body weight and serum concentrations of leptin, lipids, glucose, and insulin: A meta-analysis. Plast Reconstr Surg 151(3): 402e-411e.

GUGLIUCCI A. 2022. Biomarkers of dysfunctional visceral fat. Adv Clin Chem 109: 1-30.

HUMPHREY S, MUNAVALLI GS, YOELIN SG, FRIEDMANN DP, KAVALI CM & SANGHA S. 2022. Submental area treatment with ATX-101: relationship of mechanism of action, tissue response, and efficacy. Plast Reconstr Surg Glob Open 10(4): e4250.

IBÁÑEZ-VICENTE C, CARRATO-GOMEZ M, MECCARIELLO L, RIPANI U & BISACCIA M. 2021. Current status of localized submental fat treatment with sodium deoxicolate (ATX-101). Med Glas (Zenica) 18(1): 148-152.

ISAPS, INTERNATIONAL SOCIETY OF AESTHETIC PLASTIC SURGERY. 2020. ISAPS International survey on aesthetic/cosmetic procedures performed in 2020. https://www.isaps.org. Accessed 2024/12/02.

JEGASOTHY SM. 2018. Deoxycholic acid injections for braline lipolysis. Dermatol Surg 44(5): 757-760.

JOHNSON RJ, SEGAL MS, SAUTIN Y, NAKAGAWA T, FEIG DI, KANG DH, GERSCH MS, BENNER S & SÁNCHEZ-LOZADA LG. 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr 86(4): 899-906.

JOVANOVICH A ET AL. 2022. Deoxycholic acid and coronary artery calcification in the chronic renal insufficiency cohort. J Am Heart Assoc 11(7): e022891.

JOVANOVICH A, ISAKOVA T, BLOCK G, STUBBS J, SMITS G, CHONCHOL M & MIYAZAKI M. 2018. Deoxycholic acid, a metabolite of circulating bile acids, and coronary artery vascular calcification in ckd. Am J Kidney Dis 71(1): 27-34.

KLEIN KB, BACHELOR EP, BECKER EV & BOWES LE. 2017. Multiple same day cryolipolysis treatments for the reduction of subcutaneous fat are safe and do not affect serum lipid levels or liver function tests. Lasers Surg Med 49(7): 640-644.

KUMAR DP, ASGHARPOUR A, MIRSHAHI F, PARK SH, LIU S, IMAI Y, NADLER JL, GRIDER JR, MURTHY KS & SANYAL AJ. 2016. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet α cells to promote glucose homeostasis. J Biol Chem 291(13): 6626-6640.

LE COZ J. 2008. Mesotherapy & lipolysis: A comprehensive clinical approach, 1st ed., Singapore: Esthetic Medic Pte Limited, 197 p.

LIU M, CHESNUT C & LASK G. 2019. Overview of kybella (deoxycholic acid injection) as a fat resorption product for submental fat. Facial Plast Surg 35(3): 274-277.

MAMMUCARI M ET AL. 2020. Mesotherapy: From historical notes to scientific evidence and future prospects. ScientificWorldJournal 2020: 3542848.

MAMMUCARI M ET AL. 2021. A call to action by the italian mesotherapy society on scientific research. Drug Des Devel Ther 15: 3041-3047.

MARCOS R, MONTEIRO RA & ROCHA E. 2012. The use of design-based stereology to evaluate volumes and numbers in the liver: A review with practical guidelines. J Anat 220(4): 303-317.

MEHLEM A, HAGBERG CE, MUHL L, ERIKSSON U & FALKEVALL A. 2013. Imaging of neutral lipids by oil red o for analyzing the metabolic status in health and disease. Nat Protoc 8(6): 1149-1154.

METZGER KC, CROWLEY EL, KADLUBOWSKA D & GOODERHAM MJ. 2020. Uncommon adverse effects of deoxycholic acid injection for submental fullness: Beyond the clinical trials. J Cutan Med Surg 24(6): 619-624.

MUSKAT A, PIRTLE M, KOST Y, MCLELLAN BN & SHINODA K. 2022. The role of fat reducing agents on adipocyte death and adipose tissue inflammation. Front Endocrinol (Lausanne) 13: 841889.

NASSIR F, RECTOR RS, HAMMOUD GM & IBDAH JA. 2015. Pathogenesis and prevention of hepatic steatosis. Gastroenterol Hepatol (N Y) 11(3): 167-175.

OLDKNOW KJ, MACRAE VE, FARQUHARSON C & BÜNGER L. 2015. Evaluating invasive and non-invasive methods to determine fat content in the laboratory mouse. Open Life Sci 10: 81-88.

OREN R. 2014. Serum liver enzymes--should we count on them? Liver Int 34(2): 171-173.

POLETTO E. 2017. Lipolysis injections with sodium deoxycholate: Historical and current context. Fisioter Bras 18(3): 349-355.

RITTES PG. 2003. The use of phosphatidylcholine for correction of localized fat deposits. Aesthetic Plast Surg 27(4): 315-318.

ROSE PT & MORGAN M. 2005. Histological changes associated with mesotherapy for fat dissolution. J Cosmet Laser Ther 7(1): 17-19.

ROTUNDA AM, SUZUKI H, MOY RL & KOLODNEY MS. 2004. Detergent effects of sodium deoxycholate are a major feature of an injectable phosphatidylcholine formulation used for localized fat dissolution. Dermatol Surg 30(7): 1001-1008.

SAFARI H, KACZOROWSKI N, FELDER ML, BRANNON ER, VARGHESE M, SINGER K & ENIOLA-ADEFESO O. 2020. Biodegradable, bile salt microparticles for localized fat dissolution. Sci Adv 6(49): eabd8019.

SHARPE A, O'KEEFE M, WINDSOR K, THEOBALD J & FELDMAN R. 2022. Hemolysis after subcutaneous deoxycholic acid overdose. Am J Emerg Med 52: 268.e261-268.e262.

SIMENTAL-MENDÍA LE, RODRÍGUEZ-MORÁN M & GUERRERO-ROMERO F. 2008. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. Metab Syndr Relat Disord 6(4): 299-304.

SOETERS PB, WOLFE RR & SHENKIN A. 2019. Hypoalbuminemia: Pathogenesis and clinical significance. JPEN J Parenter Enteral Nutr 43(2): 181-193.

SZCZEPAŃSKA E & GIETKA-CZERNEL M. 2022. FGF21: A novel regulator of glucose and lipid metabolism and whole-body energy balance. Horm Metab Res 54(4): 203-211.

TREFTS E, GANNON M & WASSERMAN DH. 2017. The liver. Curr Biol 27(21): R1147-R1151.

TSCHANZ SA, BURRI PH & WEIBEL ER. 2011. A simple tool for stereological assessment of digital images: The STEPanizer. J Microsc 243(1): 47-59.

WALKER P & LEE D. 2015a. A phase 1 pharmacokinetic study of ATX-101: Serum lipids and adipokines following synthetic deoxycholic acid injections. J Cosmet Dermatol 14(1): 33-39.

WALKER PS, LEE DR, TOTH BA & BOWEN B. 2020. Histological analysis of the effect of ATX-101 (deoxycholic acid injection) on subcutaneous fat: Results from a phase 1 open-label study. Dermatol Surg 46(1): 70-77.

WALKER P, FELLMANN J & LIZZUL PF. 2015b. A phase I safety and pharmacokinetic study of ATX-101: Injectable, synthetic deoxycholic acid for submental contouring. J Drugs Dermatol 14(3): 279-287.

WANG J ET AL. 2021. DCA-TGR5 signaling activation alleviates inflammatory response and improves cardiac function in myocardial infarction. J Mol Cell Cardiol 151: 3-14.

YIN FC, SPURGEON HA, RAKUSAN K, WEISFELDT ML & LAKATTA EG. 1982. Use of tibial length to quantify cardiac hypertrophy: Application in the aging rat. Am J Physiol 243(6): H941-947.

ZARBAFIAN M & FABI SG. 2020. Off-label uses of deoxycholic acid in body contouring. Dermatol Surg 46 Suppl 1: S2-S7.

ZHANG S, ZHOU J, WU W, ZHU Y & LIU X. 2022. The role of bile acids in cardiovascular diseases: From mechanisms to clinical implications. Aging Dis 14(2): 261-282.

ZHOU W & ANAKK S. 2022. Enterohepatic and non-canonical roles of farnesoid x receptor in controlling lipid and glucose metabolism. Mol Cell Endocrinol 549: 111616.

ZUBIRÍA MG, GAMBARO SE, REY MA, CARASI P, SERRADELL MLÁ & GIOVAMBATTISTA A. 2017. Deleterious metabolic effects of high fructose intake: The preventive effect of lactobacillus kefiri administration. Nutrients 9(5): 470.

SUPPLEMENTARY MATERIAL

Figures S1 and S2.

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