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Effect of Hepatic Steatosis on Bioenergetic Function During Hepatic Ischemia-reperfusion: A Systematic Review

Michael JJ Chu*[,1,^], Anna J Dare[^,1], Adam SJR Bartlett[1-3], Anthony RJ Phillips[#,1-4] and Anthony JR Hickey[#3,4]

[1]*Department of Surgery, University of Auckland, New Zealand*

[2]*Liver Transplant Unit, Auckland City Hospital, Auckland, New Zealand*

[3]*Maurice Wilkins Centre for Biodiscovery, University of Auckland, New Zealand*

[4]*School of Biological Sciences, University of Auckland, Auckland, New Zealand*

Abstract: *Background:* The impact of hepatic steatosis on bioenergetics following hepatic ischemia-reperfusion injury (IRI) remains controversial and is associated with variable reports on its outcome. Large numbers of studies have been published examining the relationship between hepatic steatosis and cellular bioenergetics following hepatic IRI. This systematic review evaluates these studies.

Methods: An electronic search of the Medline and Embase databases (January 1946 to June 2012) was performed to select studies that reported relevant outcomes in animal models or patients with hepatic steatosis subjected to IRI.

Results: A total of 489 articles were identified, of which 63 animal studies met the predefined criteria and were included in the study. There was large variation in the type of animal model, duration and type of IRI utilized and histological description of hepatic steatosis. Bioenergetic impairments appear to increase the susceptibility of steatotic livers to IRI. The most common impairment was decreased adenosine triphosphate recovery with increased oxidative stress following IRI. Impaired mitochondrial function play a key role in the susceptibility of steatotic livers to IRI.

Conclusions: Animals with >30% hepatic steatosis have been shown to have poor outcome following IRI. Despite limitations of different experimental models and inconsistency in histological description, impaired mitochondrial function and bioenergetics appear to be important mediators in the decreased tolerance of steatotic livers to IRI. Future studies need to be consistent and clinically relevant to further improve our understanding of this issue.

Keywords: Energy metabolism, Fatty liver, Liver ischemia, Mitochondrial respiration, Oxidative phosphorylation, Reperfusion injury.

INTRODUCTION

Hepatic steatosis is the most common form of liver disease in the developed world, and is associated with diabetes, hyperlipidaemia and obesity [1]. With an increasing prevalence of metabolic syndrome [2], the incidence of hepatic steatosis is expected to rise [3]. Hepatic steatosis is reported to be associated with poor outcome in patients undergoing liver resection and those receiving steatotic donor livers during orthotopic liver transplantation (OLT) [4,5]. However, some centers have also reported excellent outcomes following OLT of severely steatotic donor livers [6,7]. The most notable issue related to hepatic steatosis and surgery is ischemia-reperfusion injury (IRI).

IRI is a major cause of liver damage during surgical procedures, in particular following liver retrieval and transplantation [8]. Donor hepatic steatosis is reportedly much less tolerant to IRI leading to graft failure [9]. Moderate-to-severe hepatic steatosis ($\geq$ 30%) is associated with higher rates of graft failure after OLT [4,9], and higher morbidity following liver resection [5].

Several different hypotheses have been proposed to explain the increased susceptibility of steatotic livers to liver injury. These include impaired hepatic microcirculation [10] and decreased intracellular energy levels [11]. Impaired microcirculation may result from intra-cellular lipid accumulation, which increases cell volume. This leads to obstruction of the adjacent sinusoidal space and impairs hepatic microcirculation [12]. The increased lipid levels in steatotic livers can also lead to lipotoxicity and promote formation of reactive oxygen species (ROS) that damages mitochondria [13]. Mitochondrial energy supply is fundamental, and the interruption of key mitochondrial processes disrupts normal cel-

*Address correspondence to this author at the Department of Surgery, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; Tel: +6421345320; Fax: +6493779656; E-mail: michaeljjc@gmail.com
^These authors contributed equally to this paper
#These authors share senior authorship

lular bioenergetics, impairs cellular function and leads to cell death [14]. Other potential hypotheses include impaired Kupffer cell function [15] and increased adhesion of leucocytes [10]. The susceptibility of steatotic livers to IRI is likely multi-factorial and further studies are required to elucidate the underlying mechanism.

While there is a significant body of literature investigating various aspects of each of these hypotheses, there is no cohesive overview of the evidence on the relationship between the degree of steatosis and recovery of mitochondrial oxidative capacity following hepatic IRI. Understanding this relationship and the functional consequences of hepatic steatosis is important to further improve outcome in patients undergoing liver resection or transplantation. The aim of this study is to systematically review the literature and provide a comprehensive resource on what is currently known about the impact of hepatic steatosis on cellular bioenergetics following hepatic IRI.

METHODS

An electronic search was performed of the Ovid Medline and Embase databases from January 1946 to June 2012 using the following MeSH headings and keywords; [Fatty liver OR hepatic steatosis OR microvesicular steatosis OR macrovesicular steatosis].mp; ischemia/ OR reperfusion injury/ OR ischemia reperfusion.mp. The search was started from January 1946 to identify potential articles as early as was possible on the Ovid Medline database. The search was limited to articles published in the English language.

The search aimed to identify all studies that reported on hepatic bioenergetic function of animals or humans with hepatic steatosis that were subjected to IRI. For the purpose of this review, bioenergetic impairment was defined as aberrations in oxidative phosphorylation and adenosine triphosphate (ATP) production processes that are central to cell metabolism. Studies were excluded if they (i) included subjects with non-alcoholic steatohepatitis rather than simple steatosis or (ii) were not original research (systematic review, narrative review, commentary or editorial). Non-alcoholic steatohepatitis was defined as steatosis with hepatocellular injury and inflammation without fibrosis [16].

Potential articles were identified using the above search strategy. Their titles and abstracts were manually screened by two reviewers (MC, AD). Eligible articles were retrieved and screened in depth for eligibility and data extracted using a standardized *pro forma*. Discrepancies were adjudicated independently by a third author (AH). Where publications used overlapping study populations, the publication with the largest number of patients/animals was selected. Information obtained included study population, severity and type of steatosis, experimental model; duration and type of IRI (partial/total, warm/cold) and markers of bioenergetic function measured.

RESULTS AND DISCUSSION

Searches in Medline and Embase identified 181 and 308 articles, respectively. After excluding duplicates and screening the abstracts, 65 articles were retrieved for evaluation. A further 19 articles were identified from searching the reference lists. Sixty-three studies met all the criteria and formed the basis of this review (Fig. **1**). One study by Nakano *et al.* examined the effect of hepatic steatosis on bioenergetics in both warm and cold IRI [17].

The study utilized a model of partial warm hepatic ischemia for investigating warm IRI whereas for cold IRI, the livers were subjected to cold ischemia followed by perfusion in an isolated liver model. There were no human studies that fulfilled the inclusion criteria. Results from the 63 studies are summarized according to the following type of IRI: partial (Table **1**) and total (Table **2**) warm IRI; cold preservation-reperfusion injury (PRI) following OLT (Table **3**) and isolated perfused model (IPM, Table **4**).

Method and Duration of Inducing IRI

Warm IRI

Thirty-six studies examined the effect of warm IRI and hepatic steatosis on bioenergetics. In human liver resection, the liver is subjected to warm ischemia (WI) when the Pringle maneuver is applied to minimize blood loss [79]. In liver transplantation, the graft is subjected to WI before organ procurement (in-situ WI) and during graft implantation (in-situ partial re-warming ischemia) [8]. In the studies reviewed, WI was performed *in-vivo* with either partial hepatic ischemia affecting ~70% of the total liver volume (n=21, Table **1**) or total hepatic ischemia (n=15, Table **2**). One study used warm storage at 37°C prior to reperfusion *via* IPM [52]. There was a significant variation in the duration of WI and reperfusion times with 33 different combinations of WI and reperfusion used in total. The most common length of partial and total hepatic ischemia was 60 (n=10, range 30–90 minutes) and 15 minutes (n=8, range 15–60 minutes), respectively. The duration of reperfusion ranged from 30 minutes to 48 hours. Thirteen of the 36 studies investigated several different time-points of reperfusion. The variability of duration of IRI limited detailed comparison amongst the studies.

Cold PRI

Twenty-eight studies examined the effect of cold PRI and hepatic steatosis on bioenergetics. Following cold ischemia, livers in 8/28 studies were reperfused *in-vivo* using an OLT model (Table **3**). Twenty studies reperfused *ex-vivo* using a normothermic liver perfusion circuit – IPM (Table **4**) while one study investigated both OLT and IPM [60]. The most common duration of cold ischemia was 24 hours (n=15 studies, range 40 minutes – 24 hours) while the most common length of reperfusion was 120 minutes (n=15, range 30 minutes – 14 days). The duration of cold ischemia was similar to the maximal duration of hepatic cold ischemia in clinical transplantation (24 hours) but the duration of reperfusion was greatly variable as there were 10 combinations each with different lengths of ischemia and reperfusion. This variability again limited the comparison or generalization of results between studies. In laboratory studies, the model of IPM was used as it allows evaluation of hepatic function, removed from the influence of other organs, undefined plasma constituents, and neural/hormonal effects [80]. However, this poses an issue as IPM does not mimic clinical transplantation where the liver is perfused with the influence of other

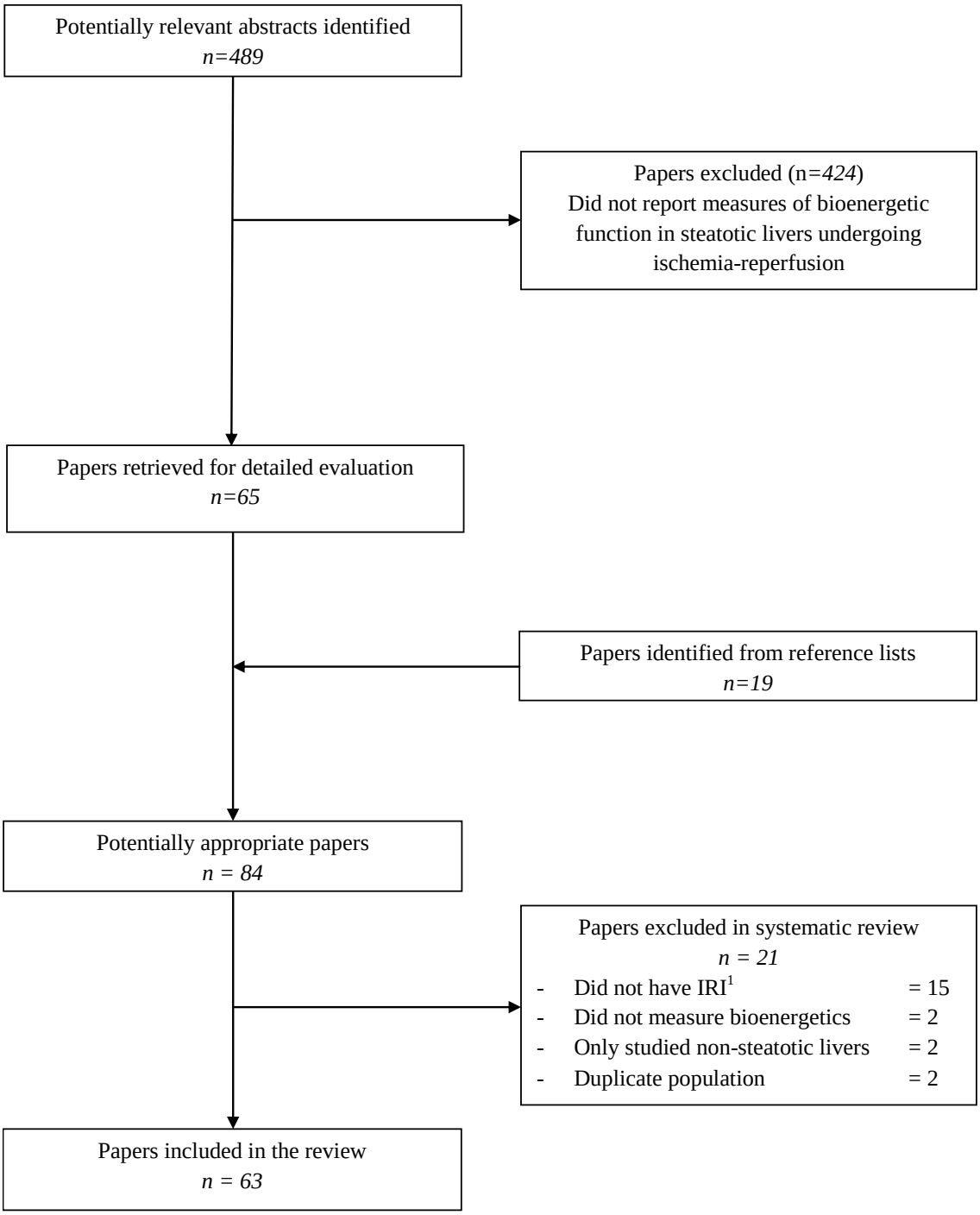

Fig. (1). QUORUM Diagram.

[1]IRI, Ischemia-reperfusion injury

organ systems and under hormonal/neural effects. The IPM model may be suitable to evaluate isolated hepatic function but does not truly reflect *in-vivo* reperfusion.

The variation in methods and IRI duration allows investigation of factors affecting outcome in steatotic livers under different settings (e.g. liver resection warm IRI vs. transplant cold PRI). We acknowledge that there are different surgical procedures and that the range of potential ischemic durations during surgery will vary, and also there is a need to mimic clinical situations in experimental studies. However, current experimental IRI models appear to be based on laboratory preferences and although the duration of WI may vary slightly (minutes) between studies, the effects on hepatic function is unknown. Therefore there is a need for a consistent duration of IRI and careful consideration of the model of IRI [81], which would permit consistent evaluation of hepatic function.

Table 1. Impact of Partial Warm Ischemia-reperfusion Injury and Hepatic Steatosis on Bioenergetics

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Koti *et al.*[a]	Rat[1]	HC	30-60	MaS	45	120	ATP	↓ATP
Hafez *et al.*[b]	Rabbit[1]	HC	40-60	MaS	60	420	CCO, CS	↓CCO, ↓CS
Tacchini *et al.*[c]	Rat	Genetic	60-70	MaS	60	120 to 24 hours	GSH, HO-1, MDA	↓GSH, ↓HO-1, ↑MDA
El-Badry *et al.*[d]	Mouse[1]	Genetic	>60	MaS	45	180	HO-1, MDA	↑HO-1, ↑MDA
Selzner *et al.*[e]	Mouse	Genetic or CDD	>60	MaS (Genetic) MiS (CDD)	45	30 to 24 hours	ATP	↓ATP
Llacuna *et al.*[f]	Mouse	CDD, HC or genetic	ns	MiS (HC) MaS (CMDD) MaS (Genetic)	90	360	GSH, MDA, MPT, ROS	↓GSH, ↑MDA, ↑MPT, ↑ROS
Sun *et al.*[g]	Rat	Genetic	>60	Mixed	40	60	MMP, Sinusoidal diameter	↓MMP
Serafin *et al.*[h]	Rat	Genetic	60-70	Mixed	60	120, 360 or 24 hours	GSH, MDA, SOD, TNF-α	↓GSH, ↑MDA
Casillas-Ramirez *et al.*[i]	Rat	Genetic	60-70	Mixed	60	24 hours	IGF-1, IGF-I:IGFBP, p38, PPAR-γ	↓IGF-1, ↓IGF-1:IGFBP, ↑PPAR-γ
Rolo *et al.*[j]	Rat	CDD	>60	Mixed	90	720	AdeNuc, ATP-Synthase, MitoResp (25°c), MMP, MPT	↓ATP, ↓ATP-Synthase, ↑State 4, ↓RCR, ↓MMP, ↑MPT
Koneru *et al.*[k]	Rat	Genetic	ns	Mixed	45 or 90	120 to 48 hours	MDA	↑MDA
Laurens *et al.*[l]	Rat	Genetic	35 ± 2.6	ns	75	360	Apoptosis, ATP	↓Apoptosis, ↓ATP
Kaneshiro *et al.*[m]	Rat	CMDD	>30	ns	60	120	ATP, GSH, GSSG	↓ATP, ↓GSH
Nakano *et al.*[n]	Rat[2]	CMDD	30-60	ns	30	720	GSH, GSSG	↓GSH
Yamagami *et al.*[o]	Rat[1]	Genetic	40-60	ns	60	120	GSH, GSSG, HO-1	↓GSH, ↑GSSG, ↓HO-1
Massip-Salcedo *et al.*[p]	Rat	Genetic	60-70	ns	60	24 hours	JNK, MDA, p38, Peroxide, PPAR-α, TNF-α	↑JNK, ↑MDA, ↑p38, ↑Peroxide, ↓PPAR-α

Table 1. Contd….

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Hong *et al.*[q]	Mouse	Genetic or HFD	>60	ns	50	360	PPAR-α, TNF- α	↓PPAR-α, ↑TNF-α
Andraus *et al.*[r]	Rat	PFD	>60	ns	60	240	MDA, MitoResp (28°c)	↑MDA, ↓P/O
Massip-Salcedo *et al.*[s]	Rat	Genetic	ns	ns	60	30 to 24 hours	HO-1, JNK, p38	↑HO-1
Selzner *et al.*[t]	Rat	Genetic	ns	ns	60	180 to 48 hours	Caspase 3 & 8, Cyt-C release	↓Caspase 3 & 8 activity, ↓Cyt-C release
Selzner *et al.*[u]	Mouse	CDD	ns	ns	75	240 or 24 hours	ATP, Caspase-3	↓ATP, ↓Caspase-3

AdeNuc, Adenine nucleotide; ATP, Adenosine triphosphate; CCO, Cytochrome c oxidase; CDD, Choline-deficient diet; CMDD, Choline-methionine deficient diet; CS, Citrate synthase; Cyt-C, Cytochrome c; GSH, Reduced gluthatione; GSSG, Gluthatione disulfide; HC, High cholesterol diet; HFD, High fat diet; HO-1, Heme-oxygenase 1; IGF-1, Insulin-like growth factor-1; IGFBP, Insulin-like growth factor binding protein; JNK, Jun-N-Terminal kinase; MaS, Macrovesicular steatosis; MDA, Malondialdehyde; MiS, Microvesicular steatosis; MitoResp, Mitochondrial respiration; Mixed, Presence of both macrovesicular and microvesicular steatosis; MMP, Mitochondrial membrane potential; MPT, Mitochondrial permeability transition; ns, Not stated; P/O, Phosphate/oxygen ratio; PPAR, Peroxisome proliferator-activated receptors; PFD, Protein-free diet; RCR, Respiratory control ratio; SOD, Superoxide dismutase; State 4, State 4 respiration; TNF-α, Tissue necrosis factor-α;

[1], No lean control group was used in the study

[2], Nakano *et al.* examined the effect of hepatic steatosis on bioenergetics in both warm and cold IRI;

a, [18]; b, [19]; c, [20]; d, [21]; e, [22]; f, [23], g, [24]; h, [25]; i, [26]; j, [27]; k, [28]; l, [29]; m, [30]; n, [17]; o, [31]; p, [32]; q, [33]; r, [34]; s, [35]; t, [36]; u, [37]

Table 2. Impact of Total Warm Ischemia-reperfusion Injury and Hepatic Steatosis on Bioenergetics

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Domenicali *et al.*[a]	Rat[1]	CDD	>30	MaS	60	120	F_1-ATP synthase, GSH, MDA, PC	↓F_1-ATP Synthase, ↓GSH, ↑MDA, ↑PC
Fiorini *et al.*[b]	Mouse[1]	Genetic	30-60	MaS	15	24 hours	ATP, GSH, UCP-2	↓ATP, ↓GSH, ↑UCP-2
Caraceni *et al.*[c]	Rat	CDD	50-60	MaS	60	30 or 120	ATP Synthase, GSH, GSSG, MDA, MitoResp (30°c), PC	↓ATP Synthase, ↓GSH, ↑GSSG, ↓MDA, ↓State 3, ↓RCR, ↑PC
Chavin *et al.*[d]	Mouse[1]	Genetic	>60	MaS	15	90 to 48 hours	ATP, UCP-2	↓ATP, ↑UCP2
Marsman *et al.*[e]	Rat	CMDD	>60	MaS	40	24 hour	TAC, TNF-α	↓TAC, ↑TNF-α
Hui *et al.*[f]	Rat	CDD	>60	MaS	30, 45 or 60	60	ATP, TAN	↓ATP, ↓TAN, ↓Energy charge

Table 2. Contd….

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Evans *et al.*[g]	Mouse	Genetic	ns	MaS	15	1 or 24 hours	ATP, MDA, UCP-2	↓ATP, ↑MDA, ↑UCP-2
Chavin *et al.*[h]	Mouse	Genetic	ns	Mixed	15	24 hours	ATP, MitoResp (37°c), MMP, UCP-2	↓ATP, ↑Proton leak, ↑State 3 & 4 (With succinate), ↑UCP-2
Luo *et al.*[i]	Mouse	HF + CMDD	<30	Mixed	15	180	NOx, TBARS, TNF-α	↑NOx, ↑TBARS, ↑TNF-α
Serviddio *et al.*[j]	Rat	HF + CMDD	>60	Mixed	60	24 hours	ATP, ATP-Synthase, Perox-ide	↓ATP-Synthase
Mosbah *et al.*[k]	Rat	Genetic	60-70	Mixed	60	24 hours	Caspase 3, Caspase 9, Caspase 12, Cyt-C release, JNK, MDA, p38, TNF- α	↓Caspase 3, ↓Caspase 9, ↓Caspase 12, ↓Cyt-c release, ↓JNK, ↑MDA, ↑p38, ↑TNF-α
Ellet *et al.*[l]	Mouse	Genetic	60-70	ns	15	24 hours	ATP, UCP-2	↓ATP, ↑UCP-2
Evans *et al.*[m]	Mouse[1]	Genetic	ns	ns	15	1 or 24 hours	ATP, GSH, UCP-2	↓ATP, ↓GSH, ↑UCP-2
Wan *et al.*[n]	Mouse	Genetic	ns	ns	15	30 to 25 hours	TNF-α, UCP-2	↑TNF-α, ↑UCP-2
Nardo *et al.*[o]	Rat	CDD	ns	ns	60	30 or 120	GSH, MDA, PC	↓GSH, ↑MDA, ↑PC

ATP, Adenosine Triphosphate; CDD, Choline-deficient diet; CMDD, Choline-methionine deficient diet; GSH, Reduced gluthatione; GSSG, Gluthatione disulfide; HF, High-fat; JNK, Jun-N-Terminal kinase; MaS, Macrovesicular steatosis; MDA, Malondialdehyde; MitoResp, Mitochondrial respiration; Mixed, Presence of both macrovesicular and microvesicular steatosis; MMP, Mitochondrial membrane potential; NOx, Nitrates/Nitrites; ns, Not stated; PC, Protein carbonyl; RCR, Respiratory control ratio; State 3, State 3 respiration; State 4, State 4 respiration; TAC, Total antioxidant capacity; TAN, Total adenine nucleotides; TBARS, Thiobarbituric acid reactive substances; TNF-α, Tissue necrosis factor-α; UCP-2, Uncoupling protein-2;
[1], No lean control group was used in the study
a, [38]; b, [39]; c, [40]; d, [41]; e, [42]; f, [43]; g, [44]; h, [45]; i, [46]; j, [47]; k, [48]; l, [49]; m, [50]; n, [51]; o, [52]

Table 3. Impact of Cold Preservation-reperfusion Injury with Orthotopic Liver Transplantation and Hepatic Steatosis on Bioenergetics

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Man *et al.*[a]	Rat[1]	Genetic	35-50	MaS	40	48 hours	ATP, EM, TNF-α, UCP-2	↓ATP, ↑Mitochondrial damage, ↑TNF-α, ↑UCP-2
Carrasco-Chaumel *et al.*[b]	Rat	Genetic	30-60	Mixed	360	240	AdeNuc, MDA, Nitrotyrosine	↑MDA, ↑Nitrotyrosine
Fernandez *et al.*[c]	Rat	Genetic	30-60	Mixed	360	240	GSH, MDA, SOD, TNF-α	↑MDA, ↓SOD
Casillas-Ramirez *et al.*[d]	Rat	Genetic	40-60	Mixed	360	240	PPAR-γ	↑PPAR-γ

Table 3. Contd….

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Jimenez-Castro *et al.*[e]	Rat[1]	Genetic	40-60	Mixed	360	240	AdeNuc, ATP, GSH, MDA, Nitrotyrosine, NOx, SOD, XDH/XOD	↓AdeNuc, ↓ATP, ↓GSH, ↑MDA, ↑Nitrotyrosine, ↑Nox, ↓SOD, ↑XOD
Uchino *et al.*[f]	Rat	Dex	ns	Mixed	ns	7 days	TNF-α, UCP-2	↑TNF-α, ↑UCP-2
Pantoflicek *et al.*[g]	Rat	CMDD	>54	ns	240	14 days	GSH	No difference
Amersi *et al.*[h]	Rat[1]	Genetic	ns	ns	240	14 days	HO-1	↓HO-1

AdeNuc, Adenine nucleotide; ATP, Adenosine triphosphate; CMDD, Choline-methionine deficient diet; Dex, High dextrose feed; EM, Electron microscopy; GSH, Reduced gluthatione; HO-1, Heme-oxygenase 1; MaS, Macrovesicular steatosis; MDA, Malondialdehyde; Mixed, Presence of both macrovesicular and microvesicular steatosis; NOx, Nitrates/Nitrites; ns, Not stated; PPAR, Peroxisome proliferator-activated receptors; SOD, Superoxide dismutase; TNF-α, Tissue necrosis factor-α; UCP-2, Uncoupling protein-2; XDH, Xanthine Dehydrogenase; XOD, Xanthine oxidase;
[1], No lean control group was used in study
a, [53]; b, [54]; c, [55]; d, [56]; e, [57]; f, [58]; g, [59]; h, [60]

Table 4. Impact of Cold Preservation-reperfusion Injury with Isolated Perfused Model and Hepatic Steatosis on Bioenergetics

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Hata *et al.*[a]	Rat[1]	FFD-C	<60	MaS	24 hours	45	ATP, EM, GLDH, MDA, OxyC	↓ATP, ↑Mitochondrial damage, ↑GLDH, ↑MDA, ↓OxyC
von Heesen *et al.*[b]	Rat	FFD-C	40-50	MaS	24 hours	60	Caspase 3, GLDH, MDA	↑Caspase 3, ↑GLDH, ↑MDA
Nardo *et al.*[c]	Rat	CDD	>60	MaS	60	60	GSH, MDA, Superoxide	↓GSH, ↑MDA, ↑Superoxide
Caraceni *et al.*[d]	Rat	CDD	>60	MaS	18 hours	30 or 120	ATP, ATP Synthase, Complex I, MitoResp (30°c)	↓ATP, ↓ATP Synthase, ↓Complex I, ↓RCR, ↓State 3
Ferrigno *et al.*[e]	Rat	Genetic	ns	MaS	360	120	NOx, TAN	↓ATP, ↓TAN, ↑NOx
Vairetti *et al.*[f]	Rat	Genetic	ns	Mixed	360	120	AdeNuc, Caspase-3, GSH, NOx, Superoxide, TNF-α	↓ATP/ADP ratio, ↑Caspase-3, ↓GSH, ↑NOx, ↑Superoxide, ↑TNF-α
Minor *et al.*[g]	Rat[1]	FFD-C	<60	Mixed	24 hours	45	EM, GLDH	↑Mitochondrial damage, ↑GLDH
Eipel *et al.*[h]	Mouse	Genetic	>60	Mixed	24 hours	120	GLDH, UCP-2	↑GLDH, ↑UCP-2
Zaouali *et al.*[i]	Rat	Genetic	60-70	Mixed	24 hours	120	HO-1, MDA, NOx, Superoxide, TNF-α	↑MDA, ↑Superoxide, ↑TNF-α

Table 4. Contd….

Author	Animal	Steatosis Model	% Steatosis	Type of Steatosis	Duration of Ischemia (mins)	Duration of Reperfusion (mins)	Parameters Assessed	Outcome
Zaouali *et al.*[j]	Rat	Genetic	60-70	Mixed	24 hours	120	Caspase 3, Caspase 9, Caspase 12, IGF-1, PPAR-γ	↓Caspase 3, ↓Caspase 9, ↓Caspase 12
Tolba *et al.*[k]	Rat[1]	FFD-C	<60	ns	24 hours	45	ATP, EM, GLDH, MDA, TAN	↓ATP, ↑Mitochondrial damage, ↑GLDH, ↑MDA, ↓TAN
Nakano *et al.*[l]	Rat[2]	CMDD	30-60	ns	24 hours	120	GSH, GSSG, MDA	↓GSH, ↓GSSG
Amersi *et al.*[m]	Rat[1]	Genetic	ns	ns	360	120	HO-1	↓HO-1
Zaouali *et al.*[n]	Rat[1]	Genetic	ns	ns	24 hours	120	GLDH, MDA, NOx, p38, TNF-α	↑GLDH, ↑MDA, ↑NOx, ↑p38, ↑TNF-α
Zaouali *et al.*[o]	Rat[1]	Genetic	ns	ns	24 hours	120	ATP, AdeNuc, GLDH, MDA	↓ATP, ↓AdeNuc, ↑GLDH, ↑MDA
Zaouali *et al.*[p]	Rat	Genetic	ns	ns	24 hours	120	GLDH, HIF-1, HO-1, NOx	↑GLDH
Mosbah *et al.*[q]	Rat	Genetic	ns	ns	24 hours	120	AdeNuc, AMPK, GLDH, MDA, NOx, Superoxide	↓AdeNuc, ↓ATP, ↑GLDH, ↑MDA, ↑Superoxide,
Mosbah *et al.*[r]	Rat	Genetic	ns	ns	24 hours	120	AdeNuc, AMPK, GLDH, HO-1, MDA, NOx	↓AdeNuc, ↓AMPK, ↓ATP, ↑GLDH, ↓HO-1, ↑MDA
Mosbah *et al.*[s]	Rat	Genetic	ns	ns	24 hours	120	GLDH, MDA, NO	↑GLDH, ↑MDA
Mosbah *et al.*[t]	Rat	Genetic	ns	ns	24 hours	120	ATP, AdeNuc, EM, MDA	↓ATP, ↓AdeNuc, ↑Mitochondrial damage, ↑MDA,

AdeNuc, Adenine nucleotide; ADP, Adenosine diphosphate; AMPK, Adenosine monophosphate-activated protein kinase; ATP, Adenosine triphosphate; CDD, Choline-deficient diet; CMDD, Choline-methionine deficient diet; Complex I, Mitochondrial complex I activity; EM, Electron microscopy; FFD-C, Fast for 2 days and fat-free diet enriched with carbohydrate; GLDH, Glutamate dehydrogenase; GSH, Reduced gluthatione; GSSG, Gluthatione disulfide; HIF-1, Hypoxia-inducible factor 1; HO-1, Heme-oxygenase 1; IGF-1, Insulin-like growth factor-1; MaS, Macrovesicular steatosis; MDA, Malondialdehyde; MitoResp, Mitochondrial respiration; Mixed, Presence of both macrovesicular and microvesicular steatosis; NO, Nitric oxide; NOx, Nitrates/Nitrites; ns, Not stated; OxyC, Oxygen consumption; PPAR-γ, Peroxisome proliferator-activated receptors-γ; RCR, Respiratory control ratio; State 3, State 3 respiration; TAN, Total adenine nucleotides; TNF-α, Tissue necrosis factor-α; UCP-2, Uncoupling protein-2;

[1], No lean control group was used in the study

[2], Nakano *et al.* examined the effect of hepatic steatosis on bioenergetics in both warm and cold IRI;

a, [61]; b, [62]; c, [63]; d, [64]; e, [65]; f, [66]; g, [67]; h, [68]; i, [69]; j, [70]; k, [71]; l, [17]; m, [60]; n, [72]; o, [73]; p, [74]; q, [75]; r, [76]; s, [77]; t, [78];

Variation in Animal Models Utilized for IRI (Table 5)

Warm IRI

Among the 36 studies, the majority were performed on rodents; rats (n=22) and mice (n=13). Hepatic steatosis was induced using Zucker *fa/fa* rats (n=11), *ob/ob* mouse (n=11), choline-deficient or choline-methionine deficient diets (n=11), high cholesterol diet (n=3), high-fat and choline-methionine deficient diet (n=2), protein-free diet (n=1) and high-fat diet (n=1). Three studies utilized more than one method of inducing hepatic steatosis [22,23,33]. A control group, receiving a standard diet, was included in 28/36 (78%) studies. Moderate-to-severe (>30% of hepatocytes contain cytoplasmic fat inclusions) steatosis was reported in 25 studies and 1 study presented with mild (<30%) steatosis. Macrovesicular steatosis was present in 13 studies, microvesicular steatosis in 2 studies and a mixed picture was reported in 9 studies. Two studies investigated both macro- and microvesicular steatosis in the experiment [22,23]. Four studies did not report on the severity of steatosis, 8 studies did not report on the type of steatosis and 6 studies did not report on both the severity and type of steatosis.

Table 5. Animal Models of Hepatic Steatosis Used

Animal (Strain)	Models of Steatosis
Rat, Zucker	Genetic, Leptin receptor abnormality[a]
Rat, Wistar	CDD / CMDD[b]
	High fat + CMDD[c]
	FFD-C[d]
	PFD[e]
	HC[f]
Rat, Sprague-Dawley	CDD[g]
	HC[h]
	FFD-C[i]
Rat, Lewis	CMDD[j]
Rat, ACI	Dextrose[k]
Mouse, *Ob/ob*	Genetic, Leptin deficient[l]
Mouse, C57	CDD[m]
	High fat + CMDD[n]
Rabbit, New Zealand white	HC[o]

CDD, Choline deficient diet; CMDD, Choline-methionine deficient diet; FFD-C, Fast for 2 days and fat-free diet enriched with carbohydrate; HC, High cholesterol; PFD, Protein-free diet

a, [20, 24-26, 28, 29, 31, 32, 35, 36, 48, 53, 55-57, 60, 65, 66, 69, 70, 72-78]; b, [17, 23, 27, 30, 40, 42, 43]; c, [47]; d, [61, 71]; e, [34]; f, [23]; g, [38, 52, 63, 64]; h, [18]; i, [62]; j, [59]; k, [58]; l, [21, 22, 33, 39, 41, 44, 45, 49-51, 68]; m, [37]; n, [46]; o, [19]

Cold PRI

All 28 studies were performed in rodents; rats (n=27) and mouse (n=1). Hepatic steatosis was induced using Zucker *fa/fa* rats (n=17), choline-deficient or choline-methionine deficient diets (n=4), 2 days of fasting followed by fat-free diet enriched with carbohydrate (n=4), dextrose-induced (n=1), and *ob/ob* mouse (n=1). A control group fed a standard diet was included in 19/28 (68%) studies. Moderate-severe steatosis was reported in 16 studies. Macrovesicular steatosis was present in 6 studies and mixed steatosis was in 10 studies. Three studies did not report on the severity of steatosis, 3 studies did not report on the type of steatosis and 8 studies did not report on both.

As the pathogenesis of hepatic steatosis is variable in each model, this raises a difficult issue in comparing results across these studies. This issue is consistent with the ongoing debate surrounding the selection of the appropriate animal model for mimicking human hepatic steatosis [82] and the choice of animal model is largely individualized to each laboratory. There are recognized difficulties in mimicking human hepatic steatosis in animal models, so several different methods for experimentally inducing steatosis have evolved over recent years. However, there remains a general lack of consistency in reporting the degree and type of steatosis induced. The inconsistency and paucity of histological descriptions among the studies (32/63 had no description of severity and/or type of steatosis) makes data interpretation and comparison difficult. Debate still remains around the utility of individual staining methods, or whether histological diagnosis is still the gold standard [83]. This has remained a major factor in the difficulty of making detailed comparisons between both experimental and clinical studies.

In practice, a wide range of steatotic livers present clinically and the lack of cohesiveness in experimental models can preclude meaningful interpretation. There remains a definite need for a consistent animal model that better mimics the majority of clinical scenarios. More importantly, there needs to be improved reporting of tissue histology as this would allow for more valid comparisons and greater general application of data in this field. Table **5** summarizes the different animal models used in these studies.

Bioenergetics, Mitochondrial Function (Table 6)

For the purpose of this review, 5 of the 63 papers measured mitochondrial respiration post-IRI but each of the studies had different models of IRI and conditions for measuring mitochondrial function (Table **6**).

Mitochondria generate 95% of the cell's ATP *via* oxidative phosphorylation [84]. This occurs as a result of electron transfer through four complexes of the electron transport system (ETS), three of these complexes (complexes I, III and IV) pump or translocate protons from the mitochondrial matrix to the inner mitochondrial membrane space to generate a proton gradient and electronic potential (Fig. **2**). The flow of protons through the F_1/F_0 ATP-synthase back into the matrix then couples the ETS to ATP synthesis. Appropriate mito-

Table 6. Summary of Mitochondrial Function, Enzyme Activity, Membrane Potential, and Permeability Transition; and Energy Status Under Different Experimental Conditions

Parameter of mitochondrial function	Findings (Temperature of mitochondrial experiment)
State 3 respiration	No difference at baseline (30°C)[a]
	↓Post-warm/cold IRI (30°C)[b]
	No difference post-warm IRI (25°C)[c]
State 4 respiration	No difference post-cold PRI (30°C)[d]
	↑Post-warm IRI (25°C)[c]
Respiratory control ratio	No difference at baseline (30°C or 37°C)[e]
	↓Post-warm/cold IRI (25°C or 30°C)[f]
Phosphate/oxygen ratio	No difference at baseline or post-warm/cold IRI (25°C or 30°C)[f]
	Trend towards ↓post-warm IRI (28°C)[g]
Oxidative capacity (FCCP-induced)	No difference post-warm IRI (25°C)[c]
Complex I activity	↓Post-cold PRI[d]
Complex IV activity	↓Post-warm IRI[h]
Citrate synthase activity	↓Post-warm IRI[h]
ATP-synthase activity	↓Post-warm/cold IRI[i]
Mitochondrial membrane potential	↓Post-warm IRI[j]
Mitochondrial permeability transition	↑Induction post-warm IRI[k]
Adenine nucleotides	Similar ATP during ischemia[l]
	↓ATP recovery[m]
	↑ADP levels post-IRI[n]
	↓ATP levels post-IRI[o]
	↓Overall energy charge post-IRI[p]
	No difference in ADP or ATP post-transplantation[q]
	No difference in ATP post-warm IRI[r]

ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; IRI, Ischemia-reperfusion injury
a, [40]; b, [40, 64]; c, [27]; d, [64]; e, [40, 45, 64]; f, [27, 40, 64]; g, [34]; h, [19]; i, [27, 38, 40, 47, 64]; j, [24, 27]; k, [23, 27]; l, [22, 43, 64]; m, [22, 43, 45, 64]; n, [27, 43, 65]; o, [22, 30, 37, 39, 41, 44, 49, 50, 53, 57, 61, 64, 66, 71, 73, 75, 78]; p, [43, 76]; q, [54]; r, [47]

chondrial function is critical for proper cellular function, and mitochondrial dysfunction has wide metabolic consequences for the cell and survival.

Electron flow and ATP synthesis can be disrupted in different pathological states [85]. Hypoxia or impaired ATP-synthase function (through lack of adenosine diphosphate, ADP, or inhibition) will result in elevation of the membrane potential, and reduce the ETS complexes and promote superoxide anion (O_2.-) release from complexes I and III [85]. There are additional interactions in hypoxic settings where O_2.- will interact with nitric oxide (NO) to form peroxynitrite (ONOO.-) and the subsequent formation of peroxide (H_2O_2) from O_2.- promotes interactions between divalent metal ions through Haber-Weiss reactions [86]. ONOO.- will react with numerous mitochondrial proteins, but it has considerable influence over complex I and depresses its activity [87]. Interactions with H_2O_2 can also drive substantial damage to mitochondrial membranes and promote mitochondrial membrane permeabilisation and consequently apoptosis [88].

Impaired mitochondrial function has been implicated as a contributory factor in the lower tolerance of steatotic livers to IRI [40]. However, the specific sites impacted within dysfunctional mitochondria have yet to be thoroughly described. Using different substrate/inhibitor protocols, researchers are now able to test the flux through different respiratory complexes within the ETS and estimate respiration efficiencies [89]. Complex I substrates are derived from tricarboxylic acid cycle dehydrogenase reactions and release reduced nicotinamide adenine dinucleotide, while complex II, which is part of the tricarboxylic acid cycle is directly fueled by

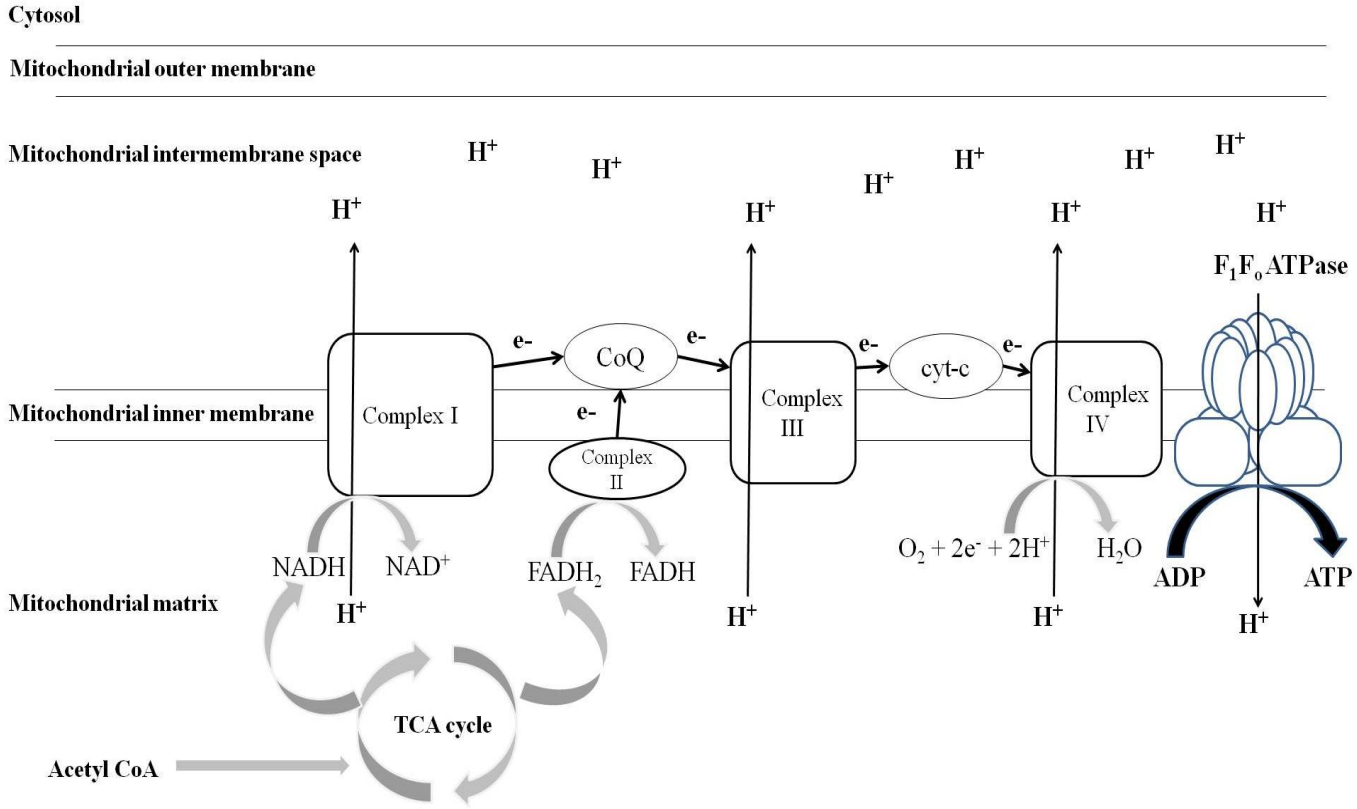

Fig. (2). Mitochondrial Electron Transport System and Oxidative Phosphorylation.

NADH and $FADH_2$ from the TCA cycle are oxidized to NAD+ and FADH by Complex I and II, respectively. The removed electrons are transferred to Coenzyme Q (CoQ) and subsequently to Complex III, Cytochrome C and Complex IV where it is transferred to an oxygen molecule to form water. Simultaneously, Complex I, III and IV translocate protons from the mitochondrial matrix to the mitochondrial intermembrane space, generating a proton gradient. The proton gradient is utilized by F_1F_o-ATPase to generate ATP from ADP. ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; CoQ, Coenzyme Q; e^-, Electron; H^+, Proton; H_20, Water molecule; FADH, Flavine-adenine dinucleotide, $FADH_2$, Reduced flavine-adenine dinucleotide; NAD+, Nicotinamide-adenine dinucleotide; NADH, Reduced nicotinamide-adenine dinucleotide; TCA, Tricarboxylic acid.

succinate. Other substrates can fuel different complexes such as electron flavoproteins and selective inhibition using specific poisons can isolate each ETS and oxidative phosphorylation complex [90]. The efficiency of respiration flux can also be estimated by testing State 4 respiration (i.e. in a non-phosphorylating respiration state) and State 3 respiration (oxidative phosphorylation or ADP-stimulated respiration [91]), and deriving a ratio termed the Respiratory Control Ratio (RCR). RCR consists of the State 3 respiration flux relative to State 4 respiration and is a measure of the degree of coupling or intactness of the mitochondrial ETS [89]. Essentially the higher or lower the ratio, the more or less respectively of overall respiration contributes to ATP synthesis. An additional measure of efficiency is the phosphate/oxygen ratio, which is an estimate of the amount of ATP formed per molecule of oxygen consumed.

Mitochondrial Function Analysis in Warm Iri

Four of the 5 studies reported on mitochondrial function following warm IRI. Only 1/4 studies reported on State 4 respiration and this was increased in steatotic livers after IRI (90 minutes partial hepatic ischemia and 12 hours reperfusion) when measured at 25°C [27]. This indicated increased proton leak across the inner mitochondrial membrane and possibly signifying increased inner membrane permeability. Similarly, Chavin *et al.* [45] also showed increased proton leak in steatotic livers pre-IRI when measured at 37°C. State 3 respiration was reported in 2 studies with conflicting results. Rolo *et al.* [27] showed no difference in state 3 respiration post-IRI between liver types whereas Caraceni *et al.* [40] showed decreased state 3 respiration in steatotic livers, indicating impaired ETS capacities in steatotic livers.

This was further shown when the RCR of steatotic liver mitochondria showed no difference to control livers at baseline pre-IRI [40,45] but showed a significant decrease post-IRI [27,40]. Interestingly, one study noted a downward trend in phosphate/oxygen ratio in post-IRI steatotic livers [34], but there was no difference in phosphate/oxygen ratio between the two groups of livers pre- or post-IRI [27,40]. There was also no difference in FCCP-induced (a mitochondrial respiratory uncoupler) respiration post-IRI indicating no difference in maximal ETS capacity between the liver types [27].

Mitochondrial Function Analysis in Cold PRI

Among the 5 studies, only one study reported on mitochondrial function following cold PRI and was in an IPM

[64]. In the study, there was no difference in state 4 respiration between liver types post-IRI when measured at 30°C but state 3 respiration was decreased in steatotic livers. However it was not statistically significant. Baseline RCR was similar between steatotic and control livers but post-IRI RCR was significantly lower in steatotic livers. There was also no difference in phosphate/oxygen ratio pre- or post-IRI between the two groups of livers.

Due to different experimental conditions within each study, it is difficult to make conclusions regarding state 3 or 4 respiration in steatotic livers and experiments conducted at sub-physiological temperatures may influence results. We note that only 1 study measured mitochondrial function at the physiological temperature of 37°C [45]. Mitochondrial respiration is temperature dependent, in particular state 4 respiration [92] and many studies use different assay temperatures ranging from 25^{o}C to 37^{o}C. Despite this, steatotic livers show a trend of decreased RCR values post-IRI, suggesting impaired mitochondrial efficiencies. This indicates that mitochondrial efficiency in steatotic liver is more greatly affected by IRI and this is supported by the presence of increased proton conductance across the inner mitochondrial membrane in steatotic livers following IRI. This may add to the susceptibility of steatotic livers to IRI. However, future mitochondrial function studies should be undertaken at the physiological temperatures to better depict mitochondrial function and to permit better comparisons among studies. This will allow greater understanding of the underlying bioenergetics and specifically mitochondrial impairment in steatotic livers.

Bioenergetics, Mitochondrial Enzyme Activities (Table 6)

Complex I and II Activity

Seven of the 63 studies measured mitochondrial enzyme activities following IRI. Complex I activity was reported in 1 study following cold PRI and was shown to be decreased in steatotic livers [64]. As complex I pumps protons across the inner mitochondrial membrane to aid development of the proton gradient, a decrease in complex I function would lead to decreased ATP generation. Chavin *et al.* [45] also showed that steatotic livers have increased complex II flux at baseline, and may indicate compensation for the decreased complex I activity. However, as complex II does not pump, it is less coupled to ATP synthesis (at only two sites, versus three sites for complex I) as it contributes substantially less to the proton gradient formation relative to oxygen flux. In essence more oxygen is consumed per ATP formed.

Cytochrome c Oxidase and Citrate Synthase Activity

Another potential explanation for the difference in mitochondrial function would be a difference in complex IV (Cytochrome C Oxidase, CCO) or citrate synthase (CS) activity. CCO reduces di-oxygen to water and pumps protons as part of the ETS whereas CS is used as a marker of mitochondrial abundance [93] as it is the entry point of the tricarboxylic acid cycle. Both enzyme activities were shown to be significantly lower in steatotic livers from New Zealand rabbits post-warm IRI when compared to sham-operated rabbits but there were no rabbits with lean livers to compare the results to [19]. These findings may partly explain the increased susceptibility of steatotic livers to IRI but thus far, no other studies have documented or compared CCO/CS activity between the two groups of livers.

ATP-Synthase Activity

Steatotic livers were also shown to have decreased activities of ATP-Synthase [27,38,40,47,64] following IRI. A decrease in ATP-Synthase function leads to decreased ATP production and would impair steatotic liver ATP synthetic capacities following IRI. In the presence of oligomycin (ATP-synthase inhibitor), Rolo and colleagues [27] showed no difference in mitochondrial respiration post-warm IRI between the two groups of livers. This suggested that the increase in resting mitochondrial flux was likely due to increased proton slip at ATP-Synthase and reaffirmed the finding of impaired ATP-Synthase function in steatotic livers.

Based on the limited number of studies, steatotic livers were shown to have decreased activities of complex I, CCO, CS, and ATP-synthase following IRI. These results support the notion that steatotic livers have impaired mitochondrial function post-IRI, especially complex I, which appears susceptible to IRI [64]. However, the exact mechanisms of the impairment are yet to be well-described. Additionally, the small number of studies that reported on complex I, CCO or CS in different IRI setting limits the generalization of the data and further studies should be repeated in different methods of IRI to allow better delineation of which enzyme is affected the most by the presence of steatosis and IRI. Despite this, steatotic liver shows impaired ATP-synthase activity following warm IRI but further investigations in cold PRI will need to be repeated to confirm the finding in the sole study that reported on ATP-synthase activity in an IPM.

Bioenergetics, Mitochondrial Membrane Analysis (Table 6)

Two of the 63 studies reported on mitochondrial membrane potential (MMP) following IRI and similarly, 2/63 studies reported on mitochondrial permeability transition (MPT). As the mitochondria require a proton gradient for efficient phosphorylation of ADP to ATP, a decrease in MMP would lead to decreased ATP production. In both studies, steatotic livers were shown to have decreased MMP [24,27] and increased lag phase (time to achieve ADP phosphorylation) [27] suggesting that steatotic livers are more susceptible to increased proton leak, impairing ATP synthesis and increasing the probability for necrosis. The 2 studies that reported on MPT also showed that steatotic livers are more susceptible to MPT induction increasing the probability of cell death [23,27].

These findings are consistent with increased proton slip at the ATP-Synthase and complex I as discussed above but also suggest that steatotic liver is more susceptible to MPT induction following warm IRI. These results also correlate with the decreased RCR observed in steatotic liver and this further depresses the capacity of steatotic livers to recover following warm IRI. However, there were no reports of MMP/MPT in cold PRI. Further studies will need to be carried out to investigate whether similar findings occur in steatotic livers following cold PRI.

Bioenergetics, Hepatic Energy Status (Table 6)

Twenty-six of the 63 papers reported on hepatic energy status post-IRI in steatotic livers. Fourteen studies were post-warm IRI and 9 studies were in an IPM while the remaining 3 studies were post-OLT. During the period or at the end of WI, ATP was low for both steatotic and lean livers [22,43]. However, the recovery of ATP levels in post-IRI steatotic livers were slower [22,43,45], with decreased ATP [22,30,37,39,41,44,49,50] and increased ADP concentrations [27,43], and the net effect of decreased overall energy charge [43] of steatotic livers. Similarly, at the end of cold ischemia, ATP levels showed no difference between steatotic and lean livers [64]. However, the recovery of ATP levels in post-cold PRI steatotic livers was reportedly slower [64], with decreased ATP [44,53,57,61,64,66,71,73,75,78] and increased ADP concentrations [65], and the net effect of decreased overall energy charge [76].

While one study reported similar levels of ATP post-warm IRI between the 2 groups of liver [47] and another reported no difference in levels of ADP and ATP between liver types post-OLT [54], the overall findings suggests that steatotic livers have decreased energy status following IRI. This is consistent with altered mitochondrial function (complex I, CCO, CS, ATP-Synthase, MMP and MPT induction) following IRI and will compromise the ability of steatotic livers to recover following the ischemic insult. These further indicate that mitochondria play key roles in the susceptibility of steatotic livers to IRI.

Mitochondrial Damage, Oxidative Stress and Antioxidants (Table 7)

Steatotic Livers have Increased Mitochondrial and Oxidative Damage following IRI

Thirty-three of the 63 studies measured mitochondrial damage and oxidative stress in steatotic livers following IRI. While mitochondria are often cited as the major sources of intracellular ROS, in health they produce little *in-vivo* [94]. However, ROS production rates increase during a number of different pathological processes, including IRI and this can promote oxidative stress and cellular damage [95]. Under such circumstances, mitochondria also become potential targets of ROS-mediated damage [96]. ROS generation in IRI also increases on reperfusion as a result of the re-introduction of oxygen. ROS damage will impact mitochondrial integrities, to further impair cellular oxygen utilization, ATP generation, and cell death with subsequent organ dysfunction and damage. In hepatic IRI, ROS production has been widely thought of as a key event for cellular damage [97,98].

Superoxide Production and Superoxide Dismutase Function

O_2.- is the initial ROS which is generated as a by-product of mitochondrial respiration. However, other enzymes such as xanthine oxidase (XOD), nicotinamide adenine dinucleotide phosphate-oxidase and monoamine oxidase [94,99] also

Table 7. Summary of Oxidative Stress, Antioxidants and Mitochondrial Damage in Steatotic Livers Following Ischemia-reperfusion Injury

Parameters Measured	Findings Post-ischemia Reperfusion Injury
Glutamate dehydrogenase	↑[a]
Superoxide (O_2^{-})	↑[b]
Hydrogen peroxide	↑[c]
Peroxynitrite	↑[d]
Xanthine oxidase	↑ (During ischemia)[e]
	↑ (During reperfusion)[f]
Lipid peroxidation	↑[g]
	No difference[h]
Protein carbonyl	↑[i]
Reduced gluthatione	↓[j]
	No difference[k]
Gluthatione disulfide	↓[l]
	↑[m]
Superoxide dismutase	↓[f]
	No difference[n]
Total antioxidant capacity	↓[o]

a, [61, 62, 67, 68, 71-77]; b, [23, 63, 66, 69, 75, 107]; c, [32, 47]; d, [54, 57]; e, [55, 57]; f, [57]; g, [20, 21, 23, 25, 28, 32, 34, 38, 40, 44, 46-48, 52, 54, 55, 57, 61-63, 69, 71-73, 75-78]; h, [17]; i, [38, 40, 52]; j, [17, 20, 23, 25, 30, 31, 38-40, 50, 52, 57, 63, 66]; k, [55, 59]; l, [17, 30]; m, [40]; n, [25, 55]; o, [42]

release O_2.-, and this can lead to generation of stronger oxidants such as hydroxyl radicals, which can damage most organic compounds [97]. During ischemia, xanthine dehydrogenase (XDH) is converted to XOD with a concurrent accumulation of its substrates, xanthine. On reperfusion, XOD reacts with xanthine and oxygen to generate O_2.- [100]. A key antioxidant, the enzyme superoxide dismutase (SOD) catalyzes O_2.- to oxygen and H_2O_2 [99], which is then degraded by catalase and glutathione peroxidase to water. However, H_2O_2 can also evolve if it escapes catalase and peroxidases and is a potent oxidant.

Peroxynitrite Production

O_2.- can also combine with NO to produce ONOO.-, a potent oxidant. ONOO.- can subtly modulate cell signaling, exert significant inhibition to most components of the ETS and trigger cell death [87]. ONOO.- has been shown to irreversibly inhibit most components of the ETS including complex I-III [101] leading to impaired oxidative phosphorylation. ONOO.- also inactivates nicotinamide nucleotide transhydrogenase, which catalyzes formation of nicotinamide adenine dinucleotide phosphate [102], and the subsequent depletion of nicotinamide adenine dinucleotide phosphate decreases the mitochondrial ability to regenerate reduced glutathione (GSH), an antioxidant. Coupled to this, another antioxidant manganese-SOD is inactivated by ONOO.-, preventing breakdown of O_2.- and further fuels oxidative damage within the mitochondria and surrounding cellular structures.

Lipid Peroxidation and Protein Carbonylation

Another example of oxidative damage is lipid peroxidation (LPO) which results from damage to lipids by ROS, and impacts cell membranes and generates reactive aldehydes [103,104] such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal. Both of these compounds react with other cellular structures, and promote further cellular damage [103,104]. LPO is commonly determined by measurement of MDA utilizing thiobarbituric acid reactive substances, which reacts with MDA to yield a fluorescent product [105]. While this is a convenient assay, the thiobarbituric acid reactive substances assay is non-specific as a marker of LPO. Therefore, the thiobarbituric acid reactive substances assay should be considered as a general marker for oxidative stress instead [104,105]. Similar to how ROS can react with lipids, ROS can also react with proteins resulting in protein oxidation and amino acid residues to be oxidized to carbonyl derivatives [97,106]. Carbonyl group formation alters protein function and subsequently decreases enzymatic activity, and makes them more susceptible to proteolytic digestion. Protein carbonyl (PC) content measurement is commonly used to estimate protein oxidation. The imbalance between the concentrations of ROS and the antioxidant defense mechanism of cells, tissue or the body, is commonly termed oxidative stress.

Glutamate Dehydrogenase and Superoxide

To assess mitochondrial damage and extent of oxidative stress in livers post-IRI, measurements have been taken of mitochondrial enzymes, LPO and ROS as markers of mitochondrial damage. Glutamate dehydrogenase is a mitochondrial enzyme and increases in glutamate dehydrogenase release were used to reflect leakage from damaged or necrotic hepatocytes [67]. Eleven studies reported on glutamate dehydrogenase and in all cases, steatotic livers showed a significant increase in glutamate dehydrogenase levels in the perfusate post-IRI indicating increased mitochondrial damage in steatotic livers [61,62,67,68,71-77].

Six studies measured levels of O_2.- and steatotic livers were shown consistently to have increased levels of O_2.- pre- [107] and post-IRI [23,63,66,69,75]. Interestingly, Nardo and colleagues [63] detected no O_2.- during ischemia but immediately after reperfusion, O_2.- was detected around the hepatic hilum. O_2.- production then occurred across the liver within minutes and peaked within 15 minutes. While there was no difference in spatial or temporal patterns of O_2.- emission, steatotic livers showed greater O_2.- emission. The combination of findings suggests that steatotic livers have greater O_2.- generation capacities at baseline and post-IRI, and that there is a progressive wave of O_2.- production across livers in general. Consistent with this, H_2O_2 was measured in 2 studies and both studies showed increased H_2O_2 levels in steatotic livers post-IRI [32,47]. Similar to these findings, 2 studies also showed increased ONOO.- levels in steatotic livers post-transplantation [54,57], further confirming increased ROS levels in steatotic livers.

Xanthine dehydrogenase/Xanthine oxidase system

The ROS-generating system of XDH and XOD were measured in 2 studies. Pre-ischemia, there was no apparent difference between steatotic and lean liver XDH/XOD activities. However, following ischemia, steatotic livers increased xanthine and XOD, indicating that steatotic livers are predisposed to generate more ROS than lean livers [55,57]. Following IRI, XOD levels in steatotic livers were approximately 90% of the total XDH/XOD activity [57]. Intravenous allopurinol (XOD inhibitor) delivery into steatotic livers post-reperfusion decreased liver injury and LPO levels following transplantation. The results from these 2 studies showed that in steatotic livers, the conversion of XDH to XOD was greater following IRI, and resulted in higher levels of ROS on reperfusion, and that XOD is a major source of ROS in steatotic livers. Currently, XOD/XDH have only been measured post-transplantation and there has yet to be a study to measure the role of XOD/XDH in steatotic livers subjected to warm IRI. Additionally, the importance of the XOD/XDH system as a major contributor of ROS in hepatic IRI is debatable [108], as some suggest the short ischemic time of most liver resections precludes XDH conversion to XOD, and therefore this system may not provide a major source of ROS. However, prolonged cold ischemia in transplantation probably provides sufficient ischemic time for conversion of XDH to XOD [108]. Moreover, the impaired microcirculation of steatotic livers may impair the flushing, and/or metabolism of xanthine/hypoxanthine on reperfusion. Therefore, while the role of XOD/XDH-mediated ROS may not be significant for normal liver resections, the XOD/XDH system may still contribute significant ROS in prolonged cold storage and for resections and transplant of steatotic liver.

Lipid Peroxidation

Twenty-seven studies utilizing the thiobarbituric acid reactive substances assay showed significantly increased levels of MDA in steatotic livers post-IRI [20,21,23,25,28, 32,34,38,40,44,46,48,52,54,55,57,61-63,69,71-73,75-78] while Nakano and colleagues [17] reported similar levels of perfusate MDA to that seen from lean livers. Serviddio *et al.* [47] also reported a significant increase in mitochondrial 4-hydroxy-2-nonenal-protein adducts post-IRI in steatotic livers. These findings suggest increased oxidative stress and LPO in steatotic livers post-IRI. The increased LPO levels in steatotic livers may be facilitated by the greater abundance of lipids in the steatotic livers, as effectively there is more substrate for the initiation and amplification of free radical generation. It has been suggested that LPO levels may not directly relate to levels of cellular injury in IRI [108]. One study reported that alanine aminotransferase increased following 60 minutes ischaemia (1-24 hours reperfusion) but LPO did not increase to the same degree as that in constantly perfused livers exposed to a pro-oxidant (*tert*-butylhydroperoxide), which did not release as much alanine aminotransferase as IRI livers [109]. However, this study did not make appropriate comparisons, as the pro-oxidant most likely produced artificially high LPO levels while in the presence of oxygen, and there was no direct comparison of the effects of the pro-oxidant in conjunction with IRI (i.e. without oxygen). Further studies into the significance of LPO in steatotic liver will be critical in underlining the relationship between LPO and cellular injury in steatotic liver.

Protein Carbonyl

Three studies measured PC levels in rodent steatotic livers and demonstrated increased levels of PC post-IRI [38,40,52]. Caraceni and colleagues found no difference in levels of hepatic PC but a significant increase in levels of hepatic mitochondrial PC [40] which was in keeping with the findings of Nardo *et al.* [52] discussed above.

Overall, steatotic livers have increased levels of ROS and mitochondrial damage post-IRI compared to lean livers. Steatotic livers have increased basal levels of O_2.- and a predisposition to generate increased levels of ROS when subjected to IRI. This leads to greater oxidative stress and damage to surrounding cellular structures.

Steatotic Livers have Decreased Antioxidant Capacity Following IRI (Table 7)

Seventeen of the 63 studies measured antioxidant capacity in steatotic livers.

Gluthatione System

One major intracellular antioxidant is GSH, which reacts with ROS and is oxidized to glutathione disulfide (GSSG) [110]. The measurement of GSH and GSSG provides a surrogate marker for the oxidative stress that the cells or organ is subjected to. Sixteen studies reported GSH levels in steatotic livers post-IRI and significantly lower GSH levels in steatotic livers was reported in 14 studies [17,20,23,25,30, 31,38-40,50,52,57,63,66] whereas the remaining 2 studies indicated similar GSH levels in both types of liver [55,59]. Three studies reported GSSG levels with one reporting increased levels of GSSG post-IRI [40]. The remaining 2 studies reported lower levels of GSSG in both hepatic tissue and perfusate of steatotic livers [17,30]. The levels of GSH in steatotic livers in these studies were all significantly lower.

Superoxide Dismutase

Three studies measured the levels of another key antioxidant, SOD. In one study SOD activities were decreased in steatotic livers post-IRI [57], yet did not differ between the groups in the other two studies [25,55]. There were no reports of SOD levels pre-IRI in either group.

Trolox

Trolox equivalent antioxidant capacity is a measurement of antioxidant strength based on Trolox (water-soluble vitamin E analogue), which serves as a standard or control antioxidant. Steatotic livers were shown to have significantly lower total antioxidant levels post-IRI as measured in Trolox [42]. These data suggest that defenses against O_2.- decrease to a greater extent in steatotic livers post-IRI and are consistent with increased hepatocellular oxidative stress.

Overall steatotic livers have decreased levels of antioxidants following IRI. However, baseline levels of antioxidants were not measured between steatotic and lean livers but have been previously reported to be similar [111]. These results further confirm that steatotic livers have a predisposition for increased ROS production rather than lacking antioxidants. It is likely that the insult of IRI would then tip the balance between ROS and antioxidants in steatotic livers leading to greater cellular susceptibility to damage, particularly to the mitochondria.

Structural Analysis (Table 8)

Electron Microscopy

Three of the 63 studies used electron microscopy to investigate mitochondrial damage post-IRI. Steatotic livers were shown to have increased mitochondrial swelling, de-

Table 8. Structural Analysis Findings from Steatotic Livers Following Ischemia-reperfusion Injury

Parameters	Findings Post-ischemia Reperfusion Injury
Electron microscopy	↑Mitochondrial swelling and damage[a]
Intravital fluorescence microscopy	↓Functional sinusoidal diameter[b]

a, [48, 53, 61, 67, 71, 78]; b, [24]

creased electro-density of matrices with less visible cristae, while control livers have better preserved mitochondrial structure [48,53,61,67,71,78].

Intravital Microscopy

Using intravital fluorescence microscopy, Sun *et al.* [24] measured sinusoidal diameter and the hepatic cord width post-IRI. While they reported no significant difference in sinusoidal diameter in steatotic livers, they did however note decreased functional sinusoidal diameter in steatotic livers, which can indicate that steatotic livers are predisposed to necrosis post-IRI.

The structural analyses findings are consistent with decreased ATP synthesis due to mitochondrial damage as seen by electron microscopy and likely caused by increased oxidative stress, which is consistent with the discussions above.

Steatotic Livers have Decreased Mediators of Apoptosis (Table 9)

There are 2 distinct types of cell injury – necrosis and apoptosis [97]. Necrosis occurs as a consequence of lethal external insult to the cell, or metabolic failure, whereas in apoptosis, the cell actively participates in its death.

Caspases

Apoptosis proceeds with the activation of specific cysteine-dependent aspartate-directed proteases, or caspases, which can be driven through events extrinsic and intrinsic to the mitochondria. The mitochondrial mediated route is activated through the release of cytochrome c [112]. The extrinsic route still harnesses mitochondria, as caspase 8 triggers mitochondria to release cytochrome c which then activates caspase 3 and 9 to subsequently trigger cell death [113]. Prolonged endoplasmic reticulum stress has also been shown to activate caspase 12, which drives apoptosis [113,114].

Only one study reported caspase 8 activity and showed lower caspase 8 activity in steatotic livers post-IRI [36]. Six of the 63 studies reported Caspase 3 activity with 4/6 studies indicated that steatotic livers have lower caspase 3 activity post-IRI [36,37,48,70] whereas Vairetti *et al.* [66] showed elevated caspase 3 activities in steatotic livers. However, the increased caspase 3 activity also coincided with signs of necrosis, increased cellular injury and ROS production [66]. Decreased caspase 9 and 12 activities were reported in 2/63 studies [48,70]. Cytochrome c release was also shown to be decreased in steatotic livers post-IRI in 2/63 studies [36,48].

Insulin-like Growth Factor

Another signal of apoptosis is insulin-like growth factor (IGF)-1 which is a hormone produced primarily by the liver and mediates multiple cellular functions including suppressing apoptosis [115]. While no difference in the levels of IGF-1 or IGF binding protein complex was apparent in baseline serum and liver tissues of both groups, IGF-1 and IGF-binding protein complex decreased more in steatotic livers than lean livers post-IRI [26]. However, others have reported no difference in IGF-1 levels of lean and steatotic animal post-IRI in an IPM [70]. These data suggests that IRI impacts IGF-1 signaling in an *in-vivo* model, and therefore may only interact with apoptotic pathways *in-vivo*.

Despite the small number of studies reporting on mediators of apoptosis, the available results indicate that steatotic livers have decreased signals for apoptosis and lead to increased proportion of cells that undergo necrosis (Table **9**).

This is consistent with the notion that increased ROS production leads to necrosis, which is the main type of cell death following IRI [108]. Necrosis is commonly assessed by histology and steatotic livers demonstrated increased necrosis on histology following IRI [37,54]. This further validates findings that steatotic livers have decreased ATP levels post-IRI compared to lean livers.

Proteins/Molecules Related to Stress/Hypoxia/Metabolism (Table 10)

There are many proteins affecting bioenergetics but their role in the susceptibility of steatotic livers has yet to be well defined.

Table 9. Mediators of Apoptosis in Steatotic Livers Following Ischemia-reperfusion Injury

Parameters Measured	Findings Post-ischemia Reperfusion Injury
Caspase 3	↑[a]
	↓[b]
Caspase 8	↓[c]
Caspase 9	↓[d]
Caspase 12	↓[d]
Cytochrome c release	↓[e]
IGF-1, IGF-1:IGF-bindng protein ratio	↓[f]
	No difference[g]

IGF, Insulin-like growth factor

a, [62, 66]; b, [36, 37, 48, 70]; c, [36]; d, [48, 70]; e, [36, 48]; f, [26]; g, [70]

Table 10. Summary of Findings of Proteins & Molecules Related to Stress/Hypoxia/Metabolism in Steatotic Livers Following Ischemia-reperfusion Injury

Parameters Measured	Findings Post-ischemia Reperfusion Injury
Uncoupling protein 2	↑[a]
Hypoxia-inducible factor-1α	No difference[b]
Heme-oxygenase 1	↑Post-warm IRI[c]
	No difference post-cold PRI[d]
	↓Post-cold PRI[e]
Nitrates/nitrites	↑[f]
	No difference[g]
Adenosine monophosphate kinase	No difference at baseline/post-IRI[h]
	↓[i]
p38	↑[j]
	No difference[k]
c-Jun-N terminal kinases	↑[l]
	No difference[m]
	↓[n]
Peroxisome proliferator-activated receptor-α	↓[l]
Peroxisome proliferator-activated receptor-γ	↑[o]
	No difference[p]
Tissue necrosis factor-α	↑Baseline levels[q]
	↑Post-IRI[r]
	No difference post-IRI[s]

IRI, Ischemia-reperfusion injury; PRI, Preservation-reperfusion injury
a, [39, 41, 45, 49-51, 53, 58, 68]; b, [74]; c, [20, 21, 31, 35]; d, [69, 74]; e, [60, 76]; f, [46, 57, 65, 66, 72]; g, [69, 74-77]; h, [54, 75]; i, [76]; j, [32, 48, 72]; k, [26, 35]; l, [32]; m, [35]; n, [48]; o, [26, 56]; p, [70]; q, [33]; r, [42, 46, 48, 51, 53, 58, 66, 69, 72]; s, [25, 32, 55];

Steatotic Livers have Increased Uncoupling Protein-2 (UCP-2) but not Hypoxia-inducible Factor 1α (HIF-1α) while Warm IRI Up-regulates Heme-oxygenase 1 (HO-1)

An example of a protein that may affect bioenergetic is UCP-2, which occurs in the mitochondrial inner membrane and mediates proton leak across the inner membrane by uncoupling substrate oxidation from synthesis of ATP, with the energy dissipated as heat [116]. Initially, UCP-2 was contended to function in thermogenesis, but more recently it has been suggested to lower ROS formation within mitochondria by dissipation of the membrane potential [117]. Nine of the 63 studies measured UCP-2 expression and in all studies, hepatic UCP-2 expression (mRNA and protein) were significantly greater in steatotic livers at baseline and at the end of reperfusion [39,41,45,49-51,53,58,68].

Another protein involved in cellular response to hypoxia is HIF-1α, which mediates genes mediating glycolysis, glucose metabolism, and oxidative damage resistance. HIF-1α is stabilized under hypoxic conditions as its α–subunit is degraded in the presence of oxygen [118]. HIF-1α induces HO-1 which protects against oxidative damage by degrading heme into iron, carbon monoxide and biliverdin, both of which may have antioxidant effects [119,120]. The reactive iron released follows detoxification pathways and also stimulates the synthesis of ferritin, promoting a secondary cellular desensitization to oxidative stress [119]. Only one study reported HIF-1α levels with no difference between the groups of liver post-IRI [74]. Eight of the 63 studies reported HO-1 levels in steatotic livers. Four studies reported a greater increase in HO-1 levels in steatotic livers post-warm IRI [20,21,31,35] whereas in cold PRI, 2 studies showed similar increase in HO-1 levels in both types of liver [69,74] and another two studies showed decreased HO-1 protein levels in steatotic livers [60,76].

Steatotic livers appear to have increased levels of UCP-2 and similar levels of HIF-1α whereas HO-1 was increased following warm IRI but the opposite effect occurred in cold PRI. The increased levels of UCP-2 may act as a compensatory mechanism to decrease ROS generation from mitochondria and account for the increased proton leak in steatotic livers while HIF-1 α and HO-1 are still to be proven as key

proteins in IRI. Both HIF-1α and HO-1 may be important mediators against oxidants but there is insufficient or inconclusive evidence to determine their true role in the setting of hepatic steatosis and IRI. However, it appears that there is a greater increase of HO-1 level in steatotic livers following warm IRI, perhaps as a counter response to oxidative stress, and occurs in lean livers as well but in the setting of cold PRI, the opposite occurs and HO-1 appears to be down-regulated. Further studies will need to be carried out to investigate the role of warm and cold PRI on the regulation of HO-1.

Role of Nitric Oxide in Steatotic Livers

While NO can react with O_2.- to form ONOO.-, NO has other physiological roles. NO can provide a protective and regulatory role in mitochondrial injury and energy metabolism [121], and NO has been shown to decrease MPT and cytochrome c release in cultured hepatocytes [122]. NO also prevents mitochondrial permeabilization following GSH depletion suggesting that in the face of oxidative stress, it can protect rather than damage cells [123]. Studies have also shown the vasodilatory effect of NO on hepatic microcirculation [124,125]. However, the balance between harm and benefit of NO depends on the site of generation, amount and transience of NO [126].

Ten of the 63 studies measured the levels of NO in the form of nitrates/nitrites. Five studies reported similar increases in nitrates/nitrites in both steatotic and lean livers post-IRI [69,74-77] while the remaining 5 studies reported a greater increase in levels of nitrates/nitrites in steatotic livers post-IRI [46,57,65,66,72]. While the role of NO in the susceptibility of steatotic livers is still controversial, when a NO-inhibitor (L-NAME) was added to the preservation solution or given to the donor animal prior to surgery, hepatic function decreased more in steatotic livers post-IRI than in lean livers [57,74,76,77].

The mechanism of NO in IRI is still debatable, but current evidence appears to indicate that steatotic livers may have a greater requirement for NO than lean livers post-IRI. The studies indicate that NO has some beneficial effect on steatotic livers following IRI and that steatotic livers appear to up-regulate nitrates/nitrites more than lean livers. This may confer protection against oxidative stress and improve hepatic microcirculation. However, sustained high levels of NO will lead to formation of ONOO.- and the regulatory mechanism of NO generation in steatotic livers could be impaired which would lead to the increased levels of ONOO.- seen (as discussed above).

Steatotic Livers have Decreased Ability to Preserve ATP Levels via Adenosine Monophosphate

Cellular depletion of ATP leads to accumulation of adenosine monophosphate which is responsible for the stimulation of adenosine monophosphate-activated protein kinase (AMPK). AMPK, a master metabolic regulator, aims at conserving ATP levels by decreasing ATP degradation as well as inducing ATP-generating systems [127]. Three of the 63 studies reported on AMPK levels in steatotic livers. One study showed baseline AMPK levels were similar between steatotic and lean livers [54]. One study reported similar increase in the levels of AMPK in both groups of livers post-IRI [75] whereas another study showed a lesser increase in levels of AMPK in steatotic livers [76].

The results appear to indicate that there is an increase in AMPK levels post-IRI in both types of liver in an attempt to compensate for the decreased levels of ATP during ischemia. Further exploring the decreased ATP levels in steatotic liver following IRI, AMPK activation may represent attempts to protect hepatocyte bioenergetics, but this may also hold true for control livers. However, steatotic livers appear to be less able to stimulate AMPK-mediated pathways and result in decreased capacities to preserve and recover ATP levels post-IRI but the findings were all reported in cold PRI. Further studies will need to examine the role of AMPK in steatotic livers subjected to warm IRI.

Potential Up-regulation of Mitogen-activated Protein Kinase in Steatotic Livers

Mitogen-activated protein kinase (MAPK) is a group of serine/threonin-specific protein kinases that responds to extracellular stimuli and regulates various cellular activities such as cell survival and apoptosis [128]. The MAPK family includes p38 MAPKs and c-Jun-N terminal kinases (JNKs, also known as stress-activated protein kinases) and MAPKs are activated by cytokines in response to cellular stress. Activation of p38 MAPKs can prevent ROS formation making it an important mediator in the inflammatory response [129]. Sustained activation of JNK in response to stress, such as ischemia, has been shown to induce apoptosis and is also considered to have anti-inflammatory properties [130]. Five of the 63 studies measured MAPK in steatotic livers subjected to IRI.

All five studies measured levels of p38 and two studies reported similar increase in levels of p38 post-IRI, likely in response to increased ROS generation [26,35]. The other 3 studies showed an increase in p38 levels in steatotic livers post-IRI [32,72] but only 1/3 studies had lean livers in the study [48]. Three of the 5 studies reported levels of JNK in steatotic livers but the results were inconsistent. One study showed no difference in levels of JNK between both types of livers post-IRI [35]. In another study by the same group, the authors described increased JNK levels in steatotic livers post-IRI but there were no control livers assayed [32] while another study observed a lower increase of JNK levels post-IRI in steatotic livers [48].

Despite the role of MAPKs in inflammation and cell survival, MAPK have not been shown to play a major role in the bioenergetics of steatotic livers in IRI. The studies indicate that steatotic livers appear to mount a similar response to IRI as lean livers with induction of MAPK and up-regulation of p38 and JNK. However, the results were inconsistent and lack comparison to lean livers. The majority (4/5) of the studies was carried out in warm IRI and further studies are warranted to examine the levels of MAPK in steatotic livers subjected to cold PRI.

Tissue Necrosis Factor-α (TNF-α) Expression is Increased in Steatotic Livers Along with Decreased Peroxisome Proliferator-activated receptor (PPAR)-α and increased PPAR-γ

PPAR are a group of nuclear receptor proteins with an essential role in the regulation of carbohydrate, lipid and

protein metabolism [131]. Two of the three subtypes have been studied in IRI: PPAR- α and PPAR-γ. PPAR-α plays an important role in fatty acid beta-oxidation and appears to be a regulator of microsomal, peroxisomal and mitochondrial energy metabolism [132]. Mice deficient in PPAR-α appear to exhibit severe hepatic steatosis likely due to severe fatty acid overload in the liver. PPAR-γ also plays an important role in lipid metabolism and has been shown to increase UCP-2 expression while depressing the expression of leptin and TNF-α [131]. TNF-α is a pro-inflammatory cytokine involved in many pathophysiological states [133]. TNF-α has been demonstrated to depress mitochondrial function *in vitro* [134], compromise hepatic energy status (measured indirectly as ketone body ratio in arterial blood) in IRI [135] and activate p38 MAPK and JNK [129].

Four of the 63 studies measured levels of PPAR and 13/63 studies reported levels of TNF-α. Only one study reported PPAR-α in steatotic livers and showed lower levels post-IRI [32]. Three of the 4 studies reported levels of PPAR-γ with 2/3 studies reporting a greater increase in PPAR-γ levels in steatotic livers post-IRI [26,56]. The remaining study showed similar PPAR-γ levels between the groups of livers post-IRI [70]. In 10/13 studies, steatotic livers were shown to have increased basal levels of hepatic and serum TNF-α [33], greater increase post-IRI in hepatic tissue [42,46,48,53,58,69,72] and serum/perfusate [51,66]. This suggests a role for TNF-α in mediating IRI and potentially affecting mitochondrial function. However, Fernandez *et al.* reported similar increases in serum TNF-α levels in both types of liver post-IRI [55] while 2 other studies reported no significant increase in levels of TNF-α in steatotic livers post-IRI [25,32].

Collectively, PPAR-α and PPAR-γ may be contributing to impaired bioenergetics in steatotic livers by decreasing fatty acid β-oxidation and increasing UCP-2 levels with subsequent decreased ATP production. This will delay recovery from IRI and lead to further cellular damage. However, steatotic livers potentially up-regulate PPAR-γ to counteract TNF-α mediated inflammation. Studies of TNF-α *in vitro* and in control livers have been shown to affect mitochondrial function previously but this has now been shown to be playing a role in steatotic livers *in vivo* [42,46,48,51,53,58, 66,69,72].

These results suggest impaired bioenergetics in steatotic livers post-IRI may be mediated by PPAR-α, PPAR-γ and TNF-α. However, the number of studies that reported on both types of PPAR in IRI was small and additional studies are required to confirm the findings of these studies.

CONCLUSION

The evidence from this systematic review indicates that animals with >30% hepatic steatosis have poor outcome following IRI. With the increasing prevalence of steatotic livers, improving our understanding of the underlying mechanism of steatotic liver susceptibility to IRI is critical. Despite variations in experimental IRI models and histological descriptions, the current literature highlighted the role of mitochondrial dysfunction in the decreased tolerance and heightened IRI seen in steatotic livers. However, fundamental knowledge of the underlying mitochondrial abnormalities in steatotic livers following IRI has yet to be fully explored. Further studies into the role of bioenergetics in the capacities of steatotic livers to withstand IRI will be needed if we are to better identify those patients at greatest risk of IRI and livers that are suitable for transplant. Moreover therapeutic interventions may be better targeted at decreasing the deleterious effects of IRI in steatotic livers in liver surgery and transplant.

ABBREVIATIONS USED

AdeNuc	=	Adenine nucleotide
ADP	=	Adenosine diphosphate
AMPK	=	Adenosine monophosphate-activated protein kinase
ATP	=	Adenosine triphosphate
CCO	=	Cytochrome C Oxidase
CDD	=	Choline-deficient diet
CMDD	=	Choline-methionine deficient diet
Complex I	=	Mitochondrial complex I activity
CoQ	=	Coenzyme Q
CS	=	Citrate Synthase
Cyt-C	=	Cytochrome c
Dex	=	High dextrose feed
e^-	=	Electron
EM	=	Electron microscopy
ETS	=	Electron transport system
FADH	=	Flavine-adenine dinucleotide
$FADH_2$	=	Reduced flavine-adenine dinucleotide
FFD-C	=	Fast for 2 days and fat-free diet enriched with carbohydrate
GLDH	=	Glutamate dehydrogenase
GSH	=	Reduced gluthatione
GSSG	=	Gluthatione disulfide
H^+	=	Proton
H_2O	=	Water
H_2O_2	=	Hydrogen peroxide
HC	=	High cholesterol diet
HFD	=	High fat diet
HIF-1	=	Hypoxia inducible factor-1
HO-1	=	Heme-oxygenase 1
IGF	=	Insulin-like growth factor
IGFBP	=	Insulin-like growth factor binding protein
IPM	=	Isolated perfused model
IRI	=	Ischemia-reperfusion injury
JNK	=	Jun-N-Terminal kinase

LPO	=	Lipid peroxidation
MAPK	=	Mitogen-activated protein kinase
MaS	=	Macrovesicular steatosis
MDA	=	Malondialdehyde
MiS	=	Microvesicular steatosis
MitoResp	=	Mitochondrial respiration
Mixed	=	Presence of both macrovesicular and microvesicular steatosis
MMP	=	Mitochondrial membrane potential
MPT	=	Mitochondrial permeability transition
NAD+	=	Nicotinamide-adenine dinucleotide
NADH	=	Reduced nicotinamide-adenine dinucleotide
NO	=	Nitric oxide
NOx	=	Nitrates/nitrites
ns	=	Not stated
O_2-	=	Superoxide anion
OLT	=	Orthotopic liver transplantation
ONOO-	=	Peroxynitrite
OxyC	=	Oxygen consumption
PC	=	Protein carbonyl
PFD	=	Protein-free diet
P/O	=	Phosphate/oxygen ratio
PPAR	=	Peroxisome proliferator-activated receptor
PRI	=	Preservation-reperfusion injury
RCR	=	Respiratory control ratio
ROS	=	Reactive oxygen species
SOD	=	Superoxide dismutase
State 3	=	State 3 respiration
State 4	=	State 4 respiration
TAC	=	Total antioxidant capacity
TAN	=	Total adenine nucleotides
TBARS	=	Thiobarbituric acid reactive substances
TCA	=	Tricarboxylic acid
TNF-α	=	Tissue necrosis factor-α
UCP-2	=	Uncoupling protein-2
XDH	=	Xanthine dehydrogenase
XOD	=	Xanthine oxidase
WI	=	Warm ischemia

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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